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Section D BOTANY

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CONTENTS

HASTENING THE GERMINATION OF *PANICUM ANTIDOTALE* RETZ.*

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ABSTRACT

(1) Experiments in hastening the germination of *Panicum antidotale* were carried out.
(2) Germination was found to be relatively insensitive to temperature, between 20° and 30°C, and to illumination, whether constant, or of short duration.
(3) Predrying the dispersal units over $CaCl_2$, or analytical H_2SO_4, somewhat increased the final germination percentage, but did not hasten germination.
(4) Pregermination was found to be very effective in hastening germination, particularly when carried out at 60°C, during 1—21 days. Units thus treated could be dried before actual sowing, without loss of the beneficial effects of the treatment.
(5) Pregermination at temperatures higher than 6°C (and up to 26°C), of one day's duration, was even more effective than at 6°C, provided no drying of the units took place before sowing.
(6) Pregermination by soaking was less effective than when carried out on top of moist filter paper, but the detrimental "soaking effects" could be removed to a large extent by drying prior to sowing.

INTRODUCTION

Panicum antidotale Retz. is a perennial summer-grass, native to India, which has been experimentally cultivated in various agricultural experiment stations in the U.S.A. (Hitchcock 1950). The species is considered a promising candidate for summer pasture under arid conditions. Pasture reseeding with this species was attempted in southern Israel but very poor emergence was obtained. However, the germinated seedlings had established very well, and made abundant growth in the following summer.

Work was therefore started in order to determine the causes of the low emergence, and to find remedies for it.

MATERIALS AND METHODS

The dispersal unit of *Panicum antidotale* is a spikelet, containing one sterile and one fertile floret, enclosed betweem two glumes (U.S.D.A. 1952, pp. 220—221 and Plate 6, No. 122).

The plant material used for the reseeding trials and for the experiments described in this paper was grown in this country, harvested in 1953, and supplied by the Israel Ministry of Agriculture. Unless otherwise stated, it was stored in stoppered glass jars, under room conditions.

* The authors wish to express their gratitude to the Ford Foundation, under a grant from which this work was carried out, and to Prof. M. Evenari and Dr. Y. Putter for their advice and criticism.

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Germination was carried out in Petri dishes, on a single layer of filter paper moistened with 3 ml tap water.

Illuminated incubators (0.5°C accuracy) were used. Daily alternating temperatures were obtained by transferring the dishes from the low to the high temperature for 8—9 hours daily. Dark germination was obtained by placing the dishes in light-tight tins.

Unless otherwise stated, one hundred dispersal units were used per lot in the experiments. Results are tabulated in mean percentages ± standard errors. The latter are given merely as indicators of the variation found between replications, and are not meant as confidence intervals for the true means. Maximal 95% confidence intervals for binomial distribution ($\pm 2\sqrt{(50\% \cdot 50\%/N)}$, where N = total sample size in any one set of conditions) are given at the bottom of the Tables.

All experiments which involved different durations of treatment were arranged so that the treatments would be completed simultaneously.

EXPERIMENTAL RESULTS

The temperature and light requirements for the germination of the dispersal units of *P. antidotale* were investigated. Duplicate lots (containing 50 units each) were germinated at various constant and daily-alternating temperature, in both light and dark. The progress of germination was followed during nine days, and the results are presented in Table I.

TABLE I

Germination of dispersal units of P. antidotale at various temperatures in light and dark. Duplicate lots per test. Date of sowing 31.5.54.

Conditions of germination		Days germination 2	4	7	9
10°/15°C alternation	Light	0	0	0	0
	Dark	0	0	0	0
20°C	Light	0	17±3	59±1	62±4
	Dark	0	29±6	59±11	62±12
26°C	Light	7±1	50±2	66±4	69±7
	Dark	15±1	52±4	58±4	58±4
30°C	Light	9±3	44±6	55±5	56±4
	Dark	14±4	30±0	36±2	36±2

Maximal confidence interval = ± 7.1%.

The results showed great variability. Nevertheless, it appears that germination was obtained only within the range 20°—30°C but that at 26° and 30°C high percentages were attained earlier than at the lower temperature (20°C constant). The effects of illumination are not very clear-cut.

Examination of the plant material had disclosed that it contained a small portion of empty units. It was thought that removal of this portion of units might raise the

germination percentage, and reduce the variability of the results. Consequently, the plant material was placed in a cloth bag, and threshed by hand. The chaff was then blown away by means of an air stream. This treatment resulted in units consisting mainly of isolated fertile florets, with some naked caryopses.

The effects of constant and daily alternating temperatures on the germination of threshed material were investigated in both light and dark. Duplicate lots were germinated at 20°, 26°, 30°, and 35°C constant temperatures, and at 10°/20°, and 20°/30°C alternating temperatures, in both light and dark. The progress of germination was followed during six days, and the results are presented in Table II.

TABLE II

Germination of threshed and cleaned dispersal units of P. antidotale at various temperatures in light and dark. Duplicate lots per test. Date of sowing 12.9.54.

Conditions of germination		Days germination 2	4	6
10°/20°C	Light	0	0	17.5±2.5
	Dark	0	0	23.0±1.0
10°/26°C	Light	0	5.5±1.5	56.0±0.0
	Dark	0	6.5±2.5	52.0±3.0
20°/30°C	Light	19.0±3.0	66.5±3.5	76.5±4.5
	Dark	19.0±1.0	62.0±1.0	75.0±1.0
20°C	Light	2.0±0.0	68.0±6.0	75.0±2.0
	Dark	4.5±1.5	65.5±0.5	75.0±2.0
26°C	Light	32.5±1.5	68.0±1.0	73.0±2.0
	Dark	37.0±2.0	71.5±1.5	73.0±2.0
30°C	Light	37.0±6.0	68.5±0.5	73.5±2.5
	Dark	49.0±4.0	62.0±2.0	65.5±3.5
35°C	Light	29.5±2.5	44.5±0.5	50.0±1.0
	Dark	28.0±5.0	45.5±1.5	48.0±1.0

Maximal confidence interval = ±7.1%.

The results in general corroborated those of the previous test, but the following may be concluded from the present data: (a) The threshing treatment proved very favourable, in reducing the variability of the results, and in increasing both the final percentages, and rate of germination. (b) Germination was relatively insensitive to light and differences between light and dark at the various temperatures are not significant. (c) The optimal germination temperature is within the range between 20° and 30°C, as far as final percentage goes. However, when rates of germination are also taken into account, the range is narrowed and optimal temperature appears to lie between 26° and 30°C. (d) Daily alternating temperatures in general proved less effective than constant ones.

Very strong effects of short illuminations upon dark germination were found in *Lythrum salicaria* (Lehmann 1918), tobacco (Kincaid 1935), and lately similar results were found in this laboratory with *Atriplex dimorphostegia* (Koller 1957), *Oryzopsis*

miliacea, and *Eragrostis curvula* (in preparation). Since the germinated seeds were counted at intervals, dark germination was interrupted several times by short periods of illumination. As this might have affected their germination, the effects of short illuminations on dark-germination had to be investigated. Six lots of threshed units were germinated at 26°C in the dark. Three of these lots were kept in constant dark, the other three being illuminated for five minutes daily by an incandescent lamp (at approximately 200 FC). The experiment was carried out twice in succession. The results are presented in Table III.

TABLE III

Effects of short illuminations on dark-germination of threshed units of P. antidotale at 26°C. Three lots per test.

Conditions of germination	Test I (7—10.2.55)	Test II (9—13.2.55)
Continuous dark	66.3±2.7	76.3±1.7
Interrupted dark	59.3±1.3	78.3±2.2

Maximal confidence interval = ± 5.8%.

The results show that short illuminations had no effect on dark germination under the conditions of the test. This means that the results in Tables I and II are valid, as far as dark germination is concerned, and hence, that germination of *P. antidotale* is indeed light-insensitive.

Favourable effects of pre-drying the dispersal units prior to germination were found in *Poa pratensis* (Pickholtz 1911), in *Ulmus americana* (Steinbauer and Steinbauer 1931), and in other species (Went and Munz 1949), and also in *Atriplex dimorphostegia* (Koller 1957) and *Panicum turgidum* (Koller 1954). It was therefore decided to try the effects of pre-drying on the subsequent germination of *P. antidotale*.

Duplicate lots (50 unthreshed units each) were wrapped in porous paper and hermetically sealed inside test tubes containing 10 g granulated $CaCl_2$ each. The test tubes were placed in incubators at 5°, 20°, and 30°C, respectively. Similar control lots were subjected to the same temperature conditions, in hermetically sealed test tubes with no $CaCl_2$. Three durations of treatment were tried (7, 14, and 21 days). Germination was subsequently carried out at 26°C in the dark, and counts made on the 2nd, 6th, and 9th days. The results are presented in Table IV.

The final (9th day) germination percentages were transformed to degrees and subjected to analysis of variance (Goulden 1952), in order to check the significance of differences between the various treatments. The results of the analysis showed that, while the differences between the various temperatures and durations of storage were not significant, the overall difference between the predried and non-predried was significant ($P<0.01$). Furthermore, predried units had a consistently higher final (9th day) percentage than the undried controls, though the individual differences were not always significant.

TABLE IV

The effects of predrying over $CaCl_2$ on the subsequent germination of dispersal units of P. antidotale. Duplicate lots (50 unthreshed units each) per test. Date of sowing 6.7.54.

Conditions of storage			Days germination		
Temp. (°C)	Duration (days)	Drying	2	6	9
	7	—	26± 1	64± 7	65± 8
		+	32± 7	68± 4	72± 2
5°	14	—	31± 5	65± 3	68± 2
		+	25±13	79±11	82±10
	21	—	32± 6	57± 8	58± 9
		+	32± 2	71± 6	84± 3
	7	—	37± 7	70± 2	70± 2
		+	25± 2	74± 2	74± 2
20°	14	—	29± 4	66± 8	69± 7
		+	23± 0	72± 5	72± 5
	21	—	33± 6	64± 8	64± 8
		+	36± 9	85± 2	86± 3
	7	—	30± 0	67± 6	69± 4
		+	16± 0	73±15	76±14
30°	14	—	22± 5	65± 1	67± 3
		+	22± 3	75± 7	81± 8
	21	—	33±10	66±10	67± 9
		+	34± 5	79± 1	83± 6

Maximal confidence interval = ± 10.0%.

The results presented in the above table also show that while slight increases in final germination percentages may be obtained by predrying the units, these increases were not in evidence during the earlier stages of germination.

Further tests were carried out in order to improve the response to predrying. In the first of these, the effects of vacuum-predrying over $CaCl_2$ were investigated. Triplicate lots (50 unthreshed units each) were predried over $CaCl_2$ in vacuum, during 30, and 76 days, at 30°, and a daily alternation of 10°/15°C, respectively. Germination was subsequently carried out at 26°C in the dark. Controls were stored for the same periods, and at the same temperatures, in hermetically sealed test tubes which did not contain $CaCl_2$. The results, which are presented in Table V, show that prolonged vacuum predrying over $CaCl_2$ was also effective in improving the germination of the units, irrespective of temperature of predrying. From the 5th day of germination onwards the longer predrying period was consistently, though only slightly, superior to the shorter one. These differences are not significant. In the second test, the effects of predrying over concentrated H_2SO_4 were compared to those

TABLE V

The effects of prolonged vacuum-predrying over $CaCl_2$ on the germination of P. antidotale at 26°C. Triplicate lots (50 unthreshed units per test). Exp. I: predried 30 days, sown — 15.7.54. Exp. II: predried 76 days, sown 31.8.54.

Storage conditions		Germination percent					
		Experiment No. I			Experiment No. II		
Temp. °C	Drying	3rd day	5th day	10th day	3rd day	5th day	9th day
10°/15°	—	45±3.2	56±4.7	60±5.1	36±4.7	58±5.8	63±5.4
	+	46±2.4	68±1.0	70±0.9	38±2.4	73±2.4	77±1.0
30°	—	39±4.4	53±7.9	56±9.9	47±0.0	66±2.0	69±3.0
	+	47±0.0	66±4.4	72±4.7	39±1.7	72±2.4	78±2.7

Maximal confidence interval = ±8.2%.

of $CaCl_2$-predrying. Dispersal units were stored over concentrated H_2SO_4 (technical), and over $CaCl_2$, in hermetically sealed containers, during 7, 14, and 21 days, respectively, at 5°, 20°, and 30°C. Duplicate lots from each treatment, and untreated controls were sown at 26°C in the dark. It was found that whereas predrying over $CaCl_2$ gave the usual slight stimulation, predrying over the acid proved very detrimental to germination. The effects of the acid predrying were compared to those of the $CaCl_2$ predrying by calculating the germination of the $CaCl_2$ predried units as 100%. Relative germination of the acid treated units is given in Table VI.

TABLE VI

Comparative effects of two methods of predrying dispersal units of P. antidotale on their subsequent germination at 26°C in the dark. Duplicate lots of unthreshed units per treatment.

$$\text{"Relative germination"} = \frac{\text{Germination\% after } H_2SO_4 \text{ predrying}}{\text{Germination\% after } CaCl_2 \text{ predrying}} \times 100$$

Conditions of storage		Relative germination on		
Temp. (°C)	Duration (days)	3rd day	6th day	9th day
5°	7	30	87	93
	14	17	43	56
	21	18	49	63
20°	7	23	67	77
	14	35	80	90
	21	17	62	75
30°	7	31	64	81
	14	15	56	69
	21	15	39	48

The results show that during the first stages of germination, units predried at various temperatures over $CaCl_2$ had germinated to a much greater percentage than those predried over the acid. In the later stages of germination, on the other hand, the germination of the acid-predried units seemed to be slowly approaching that of the other units. These results indicate that the acid predrying did not kill the embryos, only exerted some delaying action on their germination.

In order to determine the cause of the inhibitory action of the acid, a comparison was made between the effects of predrying over technical H_2SO_4, chemically pure H_2SO_4, and $CaCl_2$. The tests were carried out in two replicates, and duplicate lots were used per replication. The treatment was carried out in identical, hermetically sealed glass jars, containing 60 ml acid, or 40 g granulated $CaCl_2$ each. The units were wrapped in porous paper, and suspended over the drying medium during 14 days, at 20°C. Germination was subsequently carried out at 26°C in the dark. The results are presented in Table VII.

TABLE VII

Detrimental effects of predrying by technical H_2SO_4. Four lots of unthreshed units of P. antidotale per treatment. 14 days predrying at 20°C. Sown on 14.10.54 at 26°C in the dark.

Predrying medium	Germination on the 3rd day	5th day	7th day
$CaCl_2$, granulated	64.5±1.9	85.0±3.3	86.8±2.5
H_2SO_4, analytical	63.5±0.3	83.0±1.7	85.3±2.3
H_2SO_4, technical	2.3±0.2	15.3±2.7	24.5±3.2

Maximal confidence interval = ±5.0%.

These results show clearly that the inhibitory action of the technical acid was due to toxic fumes which it produced.

Other methods for improving the emergence of *P. antidotale* by hastening the rate of germination were tried. This could be achieved by starting germination under controlled optimal conditions, and transferring the units to the field just before sprouting. Such a treatment will henceforth be termed "pregermination" in this paper.

One approach was to let the units imbibe under conditions which are normally favourable for germination. A transfer of the imbibed units to favourable conditions might, if the method proved successful, cause a more rapid germination.

The effects of a low-temperature pregermination on subsequent germination were investigated first. Duplicate lots were sown in Petri dishes at 5°C for periods of 7, 14, and 21 days, respectively. No germination was observed anywhere at the end of the treatment. All dishes were then transferred to 26°C in the dark. Duplicate control lots of untreated units were sown at the same time. The experiment was carried out twice in succession. Since moist seeds present great difficulties in sowing under field conditions, the effects of blotting excess water from the moist units before transfer to the optimal conditions were investigated simultaneously with the first of the above mentioned experiments. The germination percentages of the above mentioned three experiments are presented in Table VIII.

The results clearly show that second day germination of all pregerminated units, whether blotted or not, exceeded by at least 100% that of the controls. Also it is clear that, under the experimental conditions, length of treatment did not affect its

TABLE VIII

The effects of pregermination at 5°C for various lengths of time on the subsequent germination of P. antidotale at 26°C in the dark.
Exp. I: 2.7.54; *Exp. II:* 30.9.54.
(*Duplicate lots per test*)

Exp. No.	Duration of treatment (days)	Germination on the 2nd day	4th day	8th day
I	0	24±4	62±4	73±7
	7	65±3	76±6	81±5
	7*	58±2	72±4	74±6
	14	65±5	74±6	78±8
	14*	49±1	61±3	68±4
	21	51±3	63±1	66±2
	21*	48±6	60±2	62±0
II	0	25±6	69±1	75±2
	7	64±3	81±3	83±1
	14	68±2	83±2	84±3
	21	69±2	83±1	85±1

Maximal confidence interval = ±7.1%.
* Blotted after treatment.

effectiveness. The blotting process seems to have somewhat decreased germination on the whole, but still, units thus treated had germinated more rapidly than the controls. The above results mean that pregerminating the units at low temperatures is very effective in hastening their subsequent germination.

Although pregermination at low temperatures was found to be effective, the method used, namely imbibing the units when they are thinly spread out, is impractical for large scale work. It was therefore decided to try to achieve similar results by carrying out pregermination with the units entirely soaked in water. Separate lots of units were each immersed in 15 ml water, in test tubes. Glass wool was inserted into the tubes to prevent any of the units from floating. For comparison, similar numbers of units were sown in Petri dishes. Six tubes, and six dishes, were placed in a 5°C incubator for 7 days, and six more of each were placed there for 14 days. At the end of the treatments, three lots from each treatment were sown directly at 26°C in the dark, and the other three were blotted, and left to dry at 30°C for 48 hours before being sown as above. Untreated controls were sown under the same conditions. The progress of germination of the pregerminated non-dried units is graphically presented in Figure 1, and the effects of drying such pregerminated units are summarized in Table IX.

The following conclusions may be drawn from these results:

(a) Dish-pregermination was again shown to be effective (cf. Table VIII).

(b) Soak-pregermination retarded subsequent germination, not only in comparison with dish-pregermination, but also with untreated controls.

(c) Duration of soaking had some influence on the inhibitory action of the soaking,

TABLE IX

The effects of drying dispersal units of P. antidotale which had been pregerminated at 5°C, in different ways, and for different periods, on their subsequent germination at 26°C in the dark. Drying at 30°C during 48 hours. Three lots (unthreshed units) per test. Undried units sown on 7.2.55.

Pregermination Type	Pregermination Duration	Drying	Germination on the 1st day	Germination on the 2nd day	Germination on the 4th day
	None	None	0.5±1.6	27.0±3.0	77.3±1.3
	7 days	—	6.0±0.5	59.3±4.1	82.0±4.5
TB*		+	0	61.0±1.0	79.3±3.0
	14 days	—	10.7±1.9	68.3±2.2	83.3±0.3
		+	0	62.0±6.1	77.3±1.3
	7 days	—	0.3±3.3	12.7±0.7	61.3±1.9
S**		+	0	52.3±1.7	69.8±2.5
	14 days	—	0.7±3.3	11.7±1.2	51.0±1.2
		+	0	47.7±2.4	64.7±1.2

Maximal confidence interval = ± 5.8%.
* On top of blotters, in Petri dish.
** Soaked, in test tubes.

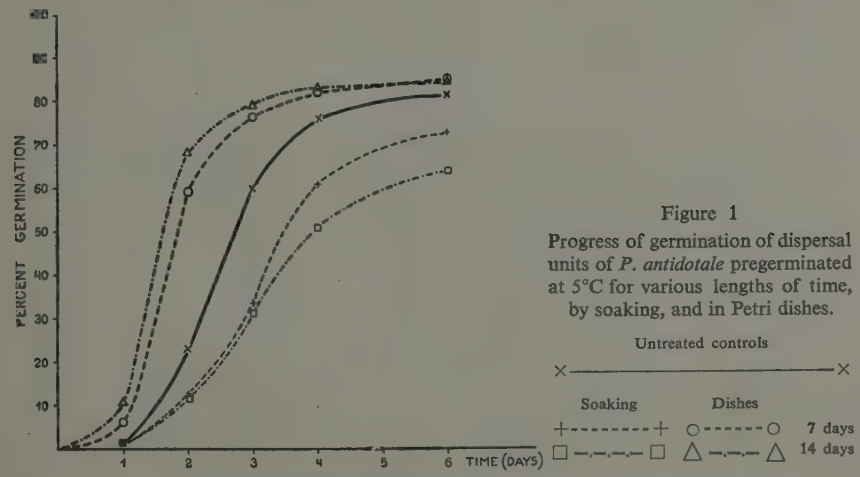

Figure 1
Progress of germination of dispersal units of *P. antidotale* pregerminated at 5°C for various lengths of time, by soaking, and in Petri dishes.

as the units soaked for seven days had germinated slightly, though consistently better than those soaked for fourteen days.

(d) Drying the dish-pregerminated units did not greatly diminish the favourable effects of pregermination, since the drying had caused only relatively small decreases in germination percentages.

(e) Drying soak-pregerminated units greatly hastened the recovery from the inhibition caused by soaking. Thus germination of units which had been soaked and

dried was much more rapid than of those which had been merely soaked, and was even faster than that of the controls. Their germination approaches, though not entirely so, that of dish-pregerminated units which had been dried.

Another pregermination treatment for hastening emergence in the field, by permitting the units to imbibe under temperatures favourable for germination for 24 hours before sowing, was also tried. Several pregermination temperatures were tried, in order to test the temperature sensitivity of such treatments, as it might have to be carried out in circumstances which do not permit temperature control (e.g. in farms or villages not possessing incubators). In several cases, drying the units between pregermination and actual sowing was also tried.

In the first experiment in these series, four lots (threshed units) were sown in Petri dishes in the dark, at —20°, +6°, +20°, +30°C constant, and at +15°/+25°C alternation, for 24 hours. Two dishes from each treatment were then transferred to 20°C in the dark, and the remaining two dishes were allowed to dry at 30°C for 24 hours, before being irrigated, and transferred to the same conditions.

Counts were made daily, during five days, and the results translated to graphical form. Final mean germination percentages P, start of germination S (representing time in days until germination had attained $P/6$), and rate of germination R (germination per day from time S, until germination had attained $5P/6$), were interpolated from the suitable graphs (according to Koller 1956). The results are tabulated in Table X.

TABLE X

Final percentage P (on 5th day), start S, and rate R, of germination at 20°C in the dark, of dispersal units of P. antidotale pregerminated at various temperatures during 24 hours, then either sown directly or after 24 hours drying at 30°C. Duplicate lots per treatment. Undried units sown on 25.2.55.

Pregermination temperature (°C)	Undried			Dried		
	P	*S*	*R*	*P*	*S*	*R*
—20°	70.0	2.1	31.1	82.0	1.6	32.2
+ 6°	75.0	1.4	31.3	88.5	1.4	39.3
+ 20°	81.0	0.8	45.0	68.5	1.6	31.5
+ 26°	83.5	0.3	39.7	72.0	1.7	24.0
+ 30°	83.0	0.3	30.7	49.0	1.6	18.7
+15°/+25°	83.0	1.2	35.7	75.0	1.4	32.3
Untreated controls	81.5	1.6	38.9	81.5	1.6	36.3

Maximal confidence interval (for P) = ± 7.1%.

From these results the following observations may be made:

(a) In undried units:

(i) Final germination percentages were independent of temperature of pregermination, higher than +6°C.

(ii) Germination started earlier with increasing temperature of pregermination. At all pregermination temperatures higher than —20°C the treatment hastened the onset of germination, as compared with the controls.

(iii) Rate of germination at 20°, and even more so at 26°C pregermination temperature, exceeded that of the controls. At all other pregermination temperatures this rate was lower than in the controls.

(b) In units which had been pregerminated, then dried:

(i) At all pregermination temperatures above +6°C, final germination percentages were lower than in the controls, particularly at 30°C.

(ii) Start of germination of all pregerminated units nearly coincided with that of the controls.

(iii) At all pregermination temperatures, except + 6°C, rate of germination of the treated units was lower than that of the controls. To both sides of this temperature the rate decreased with increasing distance from 6°C.

Since the results of the above experiment indicated that a 24 hour pregermination at 26°C without drying gave the earliest germination without loss in rate, it was decided to attempt large scale pregermination treatment at that temperature.

Large amounts of threshed units were placed in two plastic-mesh bags. One bag was dipped in a water bath (at 21°—28°C), for 20 minutes in every 70, while the other bag was left to soak undisturbed. At the start of the treatment, four lots of threshed units were pregerminated in Petri dishes, at the same temperature as that of the water bath. After 24 hours' treatment four lots from each bag were germinated at 26°C in the dark. The dish-pregerminated units, and untreated controls were simultaneously transferred to the above conditions. Counts were made on the 1st and 3rd days from sowing, and the results are presented in Table XI.

TABLE XI

The effects of pregermination by various methods on subsequent germination of P. antidotale at 26°C in the dark. Sown on 5.7.55 (*Description in text*).

Pregermination	Germination on the 1st day	Germination on the 3rd day
None (controls)	12.7±1.0	74.5±2.0
Continuous soaking	55.5±2.9	77.5±2.6
Intermittent soaking	58.0±0.2	78.5±1.1
In dishes	62.0±0.2	80.2±1.6

Maximal confidence interval = ± 5.0%.

From these results it appears that the pregermination treatment was almost equally effective in all cases. However, it was observed that on the 1st day seedling growth in all soaking treatments was much retarded in comparison with the dish pregermination. While in the former only the tip of the root-sheath had appeared by that time, in the latter both root and radicle had made considerable growth.

Pregermination by intermittent soaking for 16 seconds in every 88, and for 0.5

seconds in every second, during 24 hours, were also tried. These experiments gave essentially the same results as the above-mentioned ones.

DISCUSSION

The preliminary investigations had shown that a relatively large percentage of the dispersal units was viable, and able to germinate within a few days under a great variety of light/temperature conditions (Tables I, II and III). The low emergence percentages which had been obtained in the field could not, therefore, be attributed to low viability or other inhibitory mechanisms. It was felt that the causes for the low emergence might be due to the fact that, under desert conditions in the spring, soil moisture in the top 20—30 cm of the soil is rapidly dissipated by evaporation (Bogoslav 1955). This may have prevented the completion of germination through increased moisture tension in the soil. The crust which forms on moist loess soil while it is drying, might also be a contributing factor to the low emergence. This line of reasoning indicated that one approach to the solution of the problem might be an attempt to hasten germination as much as possible, so as to increase its chance of overtaking the deterioration of conditions in the field.

The results of the various predrying treatments (Tables IV, V, VI and VII) indicate that although the treatment had some favourable effects on germination, these effects, though significant, were relatively slight, and manifested themselves mainly in increased final percentages, not in earlier germination. It is worthy of note that though dry storage of the units proved to be beneficial for them, care should be excercised in choosing the drying medium (e.g. Table VII).

Pregermination by moist cold-storage during one to three weeks proved to be very effective in hastening germination, without noticeably affecting final percentages (Table VIII). From these results it would seem that the above treatment succeeded in doing essentially the same for *P. antidotale*, as the Levitt and Hamm (1943) treatment had done for *Taraxacum koksaghyz*. In both cases imbibition was carried out under conditions which do not permit the completion of germination (sprouting), but whereas in *Taraxacum* germination was prevented by osmotic pressure, in *Panicum* the same effect was obtained by unfavourable temperatures. This means that units thus treated would reach the field fully imbibed. The fact that a transfer to optimal germination temperatures caused prompt germination at high initial percentages makes this method a very likely one to succeed in causing rapid sprouting under field conditions. However, the moist condition of the units might present technical difficulties in sowing, and the blotting experiments (Table VIII) suggest that blotting the pregerminated units before sowing had somewhat reduced their rate of germination. The reason for this is unknown.

Later experiments (summarized in Table IX and Figure 1) were intended to answer the question of the effects of drying on the pregermination treatment. At the same time, an answer was sought to the problem of large-scale pregermination treatment. From these results it is quite clear that, though drying of pregermination units had slightly

delayed the start of their germination, most of the favourable effects of the dish-pregermination were retained. Since units thus dried were apparently as dry as the controls at time of sowing, the pregermination treatment would seem to be no mere "pre-imbibition", but rather a setting in motion of some other process which is essential for germination. This process is apparently irreversible by drying.

All the experiments hereto reported with *P. antidotale* have failed to give any indication of the presence of some chemical inhibitor in the dispersal units. This precluded the possibility that such an inhibitor is leached away during pregermination. Likewise, the possibility put forward by Levitt and Hamm (1943), that the favourable effect might be due to seed maturation in moist storage before sowing, does not seem to apply to the present case, because final percentages are not affected by the treatment. The possibility is therefore tentatively put forward that some of the favourable effects of pregermination may be attributed to mechanical causes. The swelling grains might possibly bring about a slight separation of the palea and lemma, which is irreversible by drying. This separation might facilitate subsequent entry of water, exchange of gases, or protrusion of the growing embryo.

Pregermination by soaking was shown to be detrimental to both rate, and final germination percentage (Table IX and Figure 1). The damage thus caused does not, however, seem to be of a permanent nature, because drying the units thus treated not only restored their germinability, but even caused it to approach that of the units which had been given the optimal treatment and then dried. It would seem that the soaking produces some inhibitory effects on the process of germination, which are only gradually dissipated by exposure to air. This dissipation is greatly accelerated by the drying process, and leaves the favourable after-effects of the optimal dish-pregermination.

From the results of the one-day pregermination at various temperatures (Table X) it is possible to distinguish two types of treatment. In one, pregermination takes place at temperatures which are unfavourable for the complete process of germination. Units thus treated are unaffected by drying, in start, rate, and final germination. In the other, pregermination takes place at temperatures favourable for the complete process of germination. Consequently, units thus treated are damaged by drying, and the amount of damage increases the more optimal for germination is the pre-germination temperature. These results are also very similar to those of Levitt and Hamm (1943), who found that drying damage was greater the less concentrated were the pregermination solutions.

The investigation of large-scale pregermination (by soaking) by the latter type of treatment described above, is summarized in Table XI. From the results it appears that soaking, whether constant or intermittent, hastens germination as does the dish-pregermination treatment. However, since seedling growth was appreciably retarded by both types of treatment, when compared with the latter, it would seem that it is some factor inherent to the process of soaking which is responsible for the temporary retardation of growth (compare Table IX).

PRACTICAL CONCLUSIONS

The optimal germination temperatures for *P. antidotale* lie between 20° and 26°C. It was found that pregermination was a good method for hastening germination. This treatment is relatively insensitive to temperature, and most effective when carried out on moist filter paper. One day's treatment is sufficient. However, if large scale treatment makes the above method impracticable, as it precludes the possibility of spreading the units thinly on moist filter paper, the possibility of pregerminating by soaking still exists. After such a treatment the units have to be dried before sowing, so as to remove the unfavourable after-effects of soaking.

If the sowing of moist units is technically possible, pregermination at any temperature between 6° and 26°C is effective (more so at the higher temperatures in this range). However, if such a possibility does not exist, pregermination temperatures higher than 6°C are to be avoided.

REFERENCES

1. Bogoslav, D., 1955, *Moisture regime in soils of the Negev Highlands*, Paper presented before the 48th Session of the Botanical Society of Israel.
2. Goulden, C. H., 1952, *Methods of Statistical Analysis*, 2nd ed., J. Wiley and Sons, New York.
3. Hitchcock, A. S., 1950, *Manual of the Grasses of the United States*, U.S.D.A., Miscellaneous Publications No. 200, Washington.
4. Kincaid, R. R., 1935, The effects of certain environmental factors on germination of Florida cigar wrapper tobacco seeds, *Bull. Fla. agric. Exp. Sta.*, 277.
5. Koller, D., 1954, Germination of seeds of some desert plants from Israel, *VIII Int. Bot. Congr.*, Paris.
6. Koller, D., 1957, Germination regulating mechanisms in some desert seeds. IV. *Atriplex dimorphostegia* Kar. et Kir., *Ecology*, **38**, 1—13.
7. Lehmann, E., 1918, Ueber die minimale Belichtungszeit, welche die Keimung der Samen von *Lythrum salicaria* ausloest, *Ber. dtsch. bot. Ges.*, **35**, 157—162.
8. Levitt, J. and Hamm, P. C., 1943, A method for increasing seed germination of *Taraxacum kok-saghyz*, *Plant Physiol.*, **18**, 288—293.
9. Steinbauer, C. E. and Steinbauer, G. P., 1931, Effects of temperature and desiccation during storage on germination of seeds of the American elm (*Ulmus americana L.*), *Proc. Amer. Soc. hort. Sci.*, **28**, 441—443.
10. U.S.D.A., 1952, *Manual for Testing Agricultural and Vegetable Seeds*, Agricultural Handbook No. 30, Washington.
11. Went, F. W. and Munz, P. A., 1949, A long term test of seed longevity, *El Aliso*, **2**, 63—75.

GROWTH AND PHOTOSYNTHESIS OF GLADIOLUS PLANTS GROWN UNDER VARIOUS LIGHT CONDITIONS

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ABSTRACT

Growth and assimilation rates of gladiolus plants originating from cormels and grown at different light intensities have been investigated. Total and leaf dry weight, plant height and the ratio of leaf to root dry weight increased constantly with age. Root dry weight increased only slightly with age. Moisture percentage, nitrogen content of leaves, relative growth rate and net assimilation rates on leaf weight and leaf total nitrogen bases decreased with age.

When compared with plants grown at low light intensities, plants grown in full light developed larger total and partial dry weights and their leaves had higher nitrogen content. Size of new corms, weight of cormels and the percentage of flowering plants were larger. Conversely, height of plants and the ratio of leaf to root weight were lower. Derived data did not show influence of different light intensities, apart from a peak at the time of spike stalk elongation in plots with a higher percentage of flowering plants, which happen to be high light intensity plots.

In the course of studies on the growth of gladiolus plants, the effect of various light intensities was studied under field conditions. As material, cormels were chosen since it is easy to grow them in very large numbers on a small area. Growth of plants originating from cormels has not yet been studied, although cormels are of importance as a source of corms of flowering size for export.

MATERIAL AND METHODS

Cormels of the Mansoer variety were sown in plots of 3 m^2 area each, at a rate of 500 per plot. Four different light conditions were created for 16 such plots, according to a randomized block trial. Different light intensities were produced by placing on the plots light iron frames of 200 by 150 by 170 cm covered with different fabrics. By temporarily lifting one flap, cultural practices and irrigation could be carried out.

Light intensities at the beginning of the experiment were: 100% (O), 61% (C), 45% (N), and 22% (J), obtained by leaving plots uncovered or covered by a white curtain cloth, a grayish net, one layer of jute cloth, respectively. Toward the end of the experiment the differences in light intensities in C and N plots were somewhat reduced owing to decreased transmission through the white cloth which became dirty. This may have somewhat affected the results of the last two harvests, but on the whole did not change markedly the general trends.

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Cormels were planted on March 4, 1955, in light soil. Germination occurred on the average about April 1st. Beginning May 4 and every 18—20 days till August 3, samples of 50 plants were uprooted from every plot, measured, weighed and dried. Nitrogen content of composite samples of leaves was determined by the semi-micro Kjeldahl method.

Thermograph data showed a larger range in daily air temperatures, as expected, in the open, than in covered plots. A daily range of 8°—9°C was usual in open plots during summer, while in the jute plot it reached only 4°—5°C. Other plots had intermediate ranges. Soil temperature at 5 and 10 cm depths was strikingly lower during days only in the jute plots (by 3° to 4°), while other sheds did not decrease soil temperature very much as compared with open plots.

RESULTS AND CONCLUSIONS

General trends

Apart from obvious and expected trends of increasing total weight, leaf dry weight and height, with age, there is also an increase in the ratio of leaf dry weight to root dry weight. This is due to the fact that increase in root weight is much less pronounced than increase in leaf weight (43% as compared with 656% during the period of 91 days from the first to the sixth harvests).

In the same period moisture percentage of plants decreased from 534% to 348% of dry weight or from 84% to 78% of fresh weight.

The nitrogen content of leaves in percentage of dry weight decreased from 2.68% to 1.74% during the same period, the regression equation based on all treatments being $N\% = 2.89—0.0095$ days, or an average decrease of about 0.01% per day.

The values of derived data, relative growth rate (R), net assimilation rates on leaf weight (E_W) and on total leaf nitrogen (E_N) bases (Williams 1946) decrease with age. There seems to be no question that in the present case this is due to increasing age and not to climatic factors. Decrease of assimilation rates with age is still a controversial question according to Watson (1956). Owing to decreasing nitrogen content of leaves, the decrease in E_N is somewhat less rapid than the decrease in E_W (see Table I).

TABLE I

Relative growth rates (R), net assimilation rates on dry leaf weight (E_W) and total leaf nitrogen (E_N) bases, calculated from averages of all treatments (800 plants at each harvest date) and their values in percent of initial values.

Interval	Days from germination	R g/100g/day	R%	E_W g/100g/day	E_W%	E_N g/g/day	E_N%
I	34—53	3.58	100	6.67	100	2.79	100
II	53—70	2.10	58.6	3.44	51.5	1.50	53.7
III	70—89	2.13	59.4	3.36	50.2	1.60	57.2
IV	89—109	1.32	36.9	2.20	32.9	1.15	41.2
V	109—125	0.89	24.9	1.65	24.7	0.88	31.5

Effects of different light intensities

Figure 1 shows that the rate of total dry matter accumulation decreases with decreasing illumination. The difference between the high intensity treatments (O and C) and the low intensity treatments (N and J) is significant at the 0.05 level, at the 6th harvest. The same trend is evident for leaf and root dry weight.

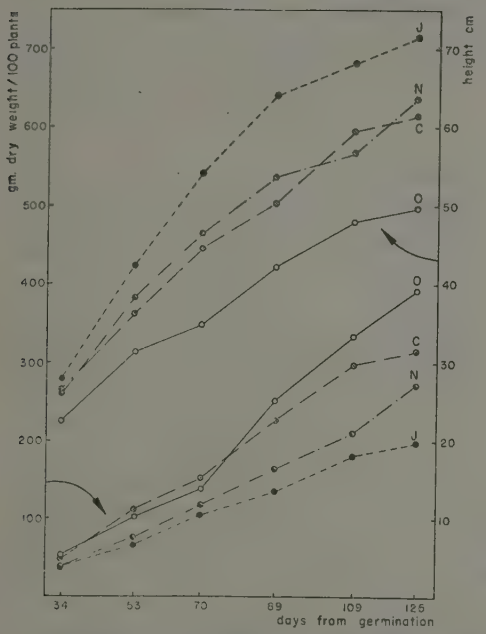

Figure 1
Total dry weight accumulation (lower set of curves) and elongation (upper set of curves) of gladiolus plants grown at different light intensities.

The ratio of leaf weight to plant height, which approximately reflects the weight of one unit length of aerial parts, also decreases with lower illumination, probably because of both narrower leaves and lower weight of leaf area. A smaller number of leaves per plant, due to slower development especially under jute cloth, may also be responsible for lower values.

Opposite trends are shown by the rate of increase in height of plants (Figure 1), which increases with decreasing illumination. The ratio of leaf to root weight follows the same trends and clearly indicates that leaf growth at low intensities is very large as compared with the amount of available roots.

The nitrogen content of leaves from the open plots was higher than that from jute plots. The difference tested by Student's method all over the period of the experiment is highly significant ($0.27\% \pm 0.067$). Other light treatments yielded intermediate values. In a previous investigation (Monselise and Heymann-Herschberg 1953) it had been shown that sun-leaves of citrus trees have higher nitrogen content than shade-leaves.

At certain dates, but not always, moisture as percentage of dry weight became higher with decreasing illumination.

The number of plants which produce a bloom has been very much affected by the intensity of light. Of 400 plants of each light treatment uprooted on the 5th and 6th harvest dates, 12.5% had flowered in open plots, as compared with 7.5%, 1.5%, 0.5% in C, N and J plots respectively.

The size of corms obtained (at the 5th and 6th harvests) is greatest in open plots and decreases with decreasing illumination (Table II). The difference between O+C vs. N+J is highly significant at the 5th harvest. The weight of cormels follows much the same pattern.

TABLE II

Circumference of new corms and dry weight of cormels of gladiolus plants grown in different light intensities.

Harvest date (days from germination)	Item	Open	Curtain	Net	Jute
109	Corms (cm)	5.3	4.8	4.3	4.2
125	Corms (cm)	5.9	5.2	4.7	4.4
125	Cormels (g/100 plants)	9.20	10.20	6.44	4.02

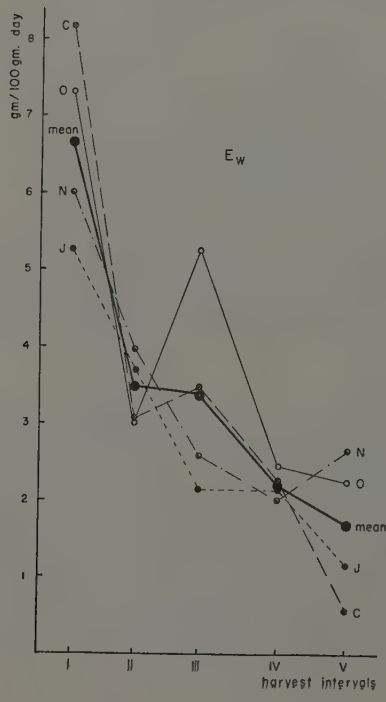

Figure 2

Net assimilation rates on leaf dry weight basis of gladiolus plants growing at different light intensities.

Derived data do not show influence of different light intensities. The peak in R, E_W, and E_N curves in open plots (see Figure 2) during the 3rd harvest interval is not significantly higher than the corresponding depression in jute plots, during the same period, although the difference is near the 0.05 level of significance. The third period includes flower spike elongation, which occurred, as already pointed out, in more than 12% of open plots plants, and was almost absent in jute plots plants. A correlation of $+0.908^{**}$ has been found between the number of flowers in plots O and C and values of R during the third interval. A similar correlation exists for values of E_W and E_N.

Peaks in growth rates corresponding to two different stages in flowering of corn have been found by Briggs, Kidd and West, and Wittwer (1943) has related them to increased hormone production at synapsis and syngamy. He also points out that in the case when male and female flowers appear together and the interval between synapsis and syngamy is shortened, only one peak may be expected.

The peak in growth curves and the high correlation found above seem to allow the conclusion that growth and assimilation of gladiolus are increased at the time of active elongation of the inflorescence. Maximum flower stalk elongation of male spinach has been shown by Wittwer (1943) to be concomitant with synapsis.

The fact that in general values of E_W and E_N are not significantly lower in plots receiving low illumination than in those thriving in full sun, may be due to the fact that the area subtending the unit of dry weight of etiolated or semi-etiolated leaves is larger than in normal plants. Net assimilation rates calculated on leaf area basis would be more suitable, in this case, in order to detect differences in leaf activity.

Some considerations on the variability of plant material involved and the limitations of growth analysis seem appropriate. A sampling unit of 50 plants with four replicates in each treatment, seems, a priori, to be a fairly large sample. Nevertheless, even larger samples are needed to overcome the variability of material and to assess differences due to illumination. The coefficient of variability for total dry weight at the 6th harvest, e.g., calculated from the analysis of all 16 plots, is 22%, although each sample consists of 50 plants. When calculating derived data this variability is even more evident since the variances of R and E are functions of the pooled variances of two successive harvests. This drawback of growth analysis has been recently reaffirmed by Watson (1956).

In the case of plants originating from cormels, the variability in results is undoubtedly increased by the lack of uniformity in emergence and by the fact that only a certain percentage of plants produce flowers.

Our experiment seems to indicate that gladiolus plants growing from cormels produce in the open a greater amount of dry matter, larger corms and a larger percentage of flowers, than at any other light intensity, ranging between 20% and 60% of full daylight. This is rather surprising when considering the very high light intensities obtaining in Israel during summer months.

REFERENCES

1. Monselise, S. P. and Heymann-Herschberg, Lotte, 1953, Influence of exposure and age on dry matter content, area and mineral composition of Shamouti orange leaves, *Proc. Amer. Soc. hort. Sci.*, **62**, 67—74.
2. Watson, D. J., 1956, Measurement of photosynthesis in field conditions, *Overdr. uit Landb. Tijdsc.*, **68**, 581—589.
3. Williams, R. F., 1946, The physiology of plant growth with special reference to the concept of net assimilation rate, *Ann. Bot. N. S.*, **10**, 41—72.
4. Wittwer, S. H., 1943, Growth hormone production during sexual reproduction of higher plants, *Univ. Mo. Agr. Exp. Sta. Res. Bul.*, 371.

EFFECT OF LENGTH OF DAY AND TEMPERATURE ON THE DEVELOPMENT OF SOME ANNUAL LEGUMES INDIGENOUS IN ISRAEL

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ABSTRACT

(1) The effects of different photoperiods and of two highly divergent temperature ranges on flowering and on some aspects of vegetative growth were studied in eighteen native leguminous annuals.

(2) In the great majority of the species tested, onset of flowering was significantly advanced as the length of photoperiod was increased by 3-hour stages from 9 to 18 hours. Within this range the photoperiodic response was of a purely quantitative nature, and there was no indication of a critical threshold. There is also some evidence that the seasonal increase in daylength may promote the onset of flowering, this effect being distinct from and additional to that of fixed photoperiods.

(3) The onset of flowering was speeded up by high temperature in all the species tested.

(4) The effect of daylength on the number of inflorescences lacked consistency, except for a certain suppression under a short photoperiod in species of *Trifolium*.

(5) Some data are supplied concerning the effect of photoperiod on axial elongation and branching. It is suggested that the extent of growth may be determined by interaction of a number of divergent trends, such as a direct morphogenetic effect, differences in the daily net assimilation rate, duration of the vegetative period, etc.

(6) With the exception of *Tetragonolobus palaestinus* in which growth was decisively enhanced by high temperature, the final extent of vegetative development tended to be much greater in plants grown at low temperature.

INTRODUCTION

Very few methodical studies have been made on the response of Palestinian plants to environmental factors. The effect of photoperiod on the development of native plants and the part played by the length of day in the geographical and seasonal distribution of the Palestinian flora is altogether unknown.

An insight into the physiological responses of native legumes is particularly desirable in view of the potential value of some of these plants in reseeding programmes concerned with the improvement of natural pasture. The fact that some legumes of the Eastern Mediterranean region have been extensively employed in selection work in other parts of the world imparts a more universal importance to the study of the

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physiological properties of our native species. Work carried out overseas is usually based on selected varieties which frequently deviate considerably from type in their responses to enviromental influences. It is therefore particularly interesting to ascertain the behaviour of plant material characterized by the original genetical make-up.

MATERIALS AND METHODS

In view of the lack of any previous experimental work on the responses of Israel legumes to environmental factors, it was considered desirable to include a rather wide range of species, reasonably representative of the family as a whole. No attempt was therefore made, at this stage, to bring in species of special economic importance, and the choice was largely determined by the availability of seed in sufficient quantity. It was endeavoured, however, to include a fair range of genera and to have the more important ones represented by a number of species. The final selection was confined to those species in which satisfactory germination had been secured in preliminary tests with scarified seed.

The eighteen species included in the experiments represent four tribes of the family Papilionaceae: Trifolieae, Loteae, Galegeae, and Vicieae. The genera *Trigonella*, *Medicago* and *Trifolium* are each represented by more than one species. Of the eight species of *Trifolium* included, six belong to the section Lagopus (*T. lappaceum*, *T. stellatum*, *T. purpureum*, *T. palaestinum*, *T. scabrum*, *T. vavilovi*) and two to the section Vesicastrum (*T. spumosum*, *T. xerocephalum*).

Carefully scarified seeds were sown outdoors on October 21, 1953, in wooden trays containing a finely sifted medium loam mixture. The seeds were spread thinly in rows on the surface of the soil and then covered with a layer of sand to approximately twice the height of the seed. Germination was recorded daily, and the date on which it had attained half the final germination percentage was fixed, for purposes of computation, as the date of germination for any given species.

Between the 9th and 11th December, when the majority of the seedlings were big enough for convenient handling, thirty seedlings of each species, selected for uniformity, were planted in well drained pots in a mixture containing three parts of medium loam to one part of old cow manure. The potted plants were divided at random into six treatment lots of five plants each. Five lots were grown on in a heated greenhouse in which the temperature was maintained thermostatically above 14°C. Uniform cubicles erected on a gravel-filled bench provided fixed photoperiods of 9, 12, 15, and 18 hours, and a natural daylength control. To minimize the effect of transitions from daylight to artificial illumination and to make the natural daylength control comparable as nearly as possible with the fixed photoperiod treatments, supplemental illumination averaging about 320 foot-candles at the tops of the plants was maintained in each cubicle for the full run of the photoperiod by the use of five 40-Watt 'daylight' fluorescent tubes attached to an adjustable, vertically sliding frame. The black-out outfit consisted of sliding, light-tight black curtains. Air

circulation between the cubicles was ensured during the black-out hours by means of small, low-voltage tubular ventilators inserted in the partition walls. Daylight was admitted to all the cubicles between 8 a.m. and 5 p.m. Photoperiods longer than 9 hours were made up by appropriate supplementation with the artificial light, by means of automatic switches. The switch attached to the control cubicle was regularly adjusted to put out the lights at sunset. With very few sporadic exceptions, temperature differences between the cubicles, as recorded by minimum-maximum thermometers, did not exceed 1°C.

In addition to the photoperiodic treatments in the greenhouse, a parallel outdoor series, comparable with the greenhouse control in respect of day length, was exposed to considerably lower temperature. The differences were particularly pronounced in the night temperature levels, outdoor temperatures being frequently close to zero. In view of the fact that reduction in light intensity under glass was largely compensated by the supplementary daytime illumination, we are reasonably justified in assuming that the discrepancies in temperature levels constitute by far the most essential difference between the outdoor and indoor control series.

The natural daylength which was 11 hrs 15 min. at the time of sowing, diminished at first down to the annual minimum of 10 hrs 25 min. (latitude of Jerusalem 31° 46.9′ N), and then went up again to just under 12 hours by the time all flowering was over on the greenhouse plants. Observations on the outdoor plants were continued until May 1, when the length of day was 13 hrs 25 min.

All the experimental plants were generously watered throughout the growing period in order to prevent any effects due to a moisture deficit. Sulphur dustings and sprays of a 5 percent D.D.T. solution were applied at frequent intervals. The pots were rearranged periodically to equalize positional effects.

The study was primarily concerned with flowering responses, but some observations were also made on the vegetative development. All data presented, other than those on the time of flowering, refer to records taken at the completion of flowering.

The effect of photoperiod was tested in the first place by analysis of variance according to two-way classification, the factors being species, photoperiod, and interaction between these two. Where interaction was found to be significant, the effect of photoperiod was analysed for each species independently.

Means which did not differ significantly were bracketed into homogeneous groups by the methods proposed by Tukey (1949) and Duncan (1955). Each group has been represented by its mean and confidence limits. The level of significance adopted for the tests is $P = 0.05$, unless otherwise stated.

The effect of temperature was tested by computation of the lowest significant difference between the treatment means for each species separately. When standard errors of treatment means were not significantly different, a common *l.s.d.* criterion was applied.

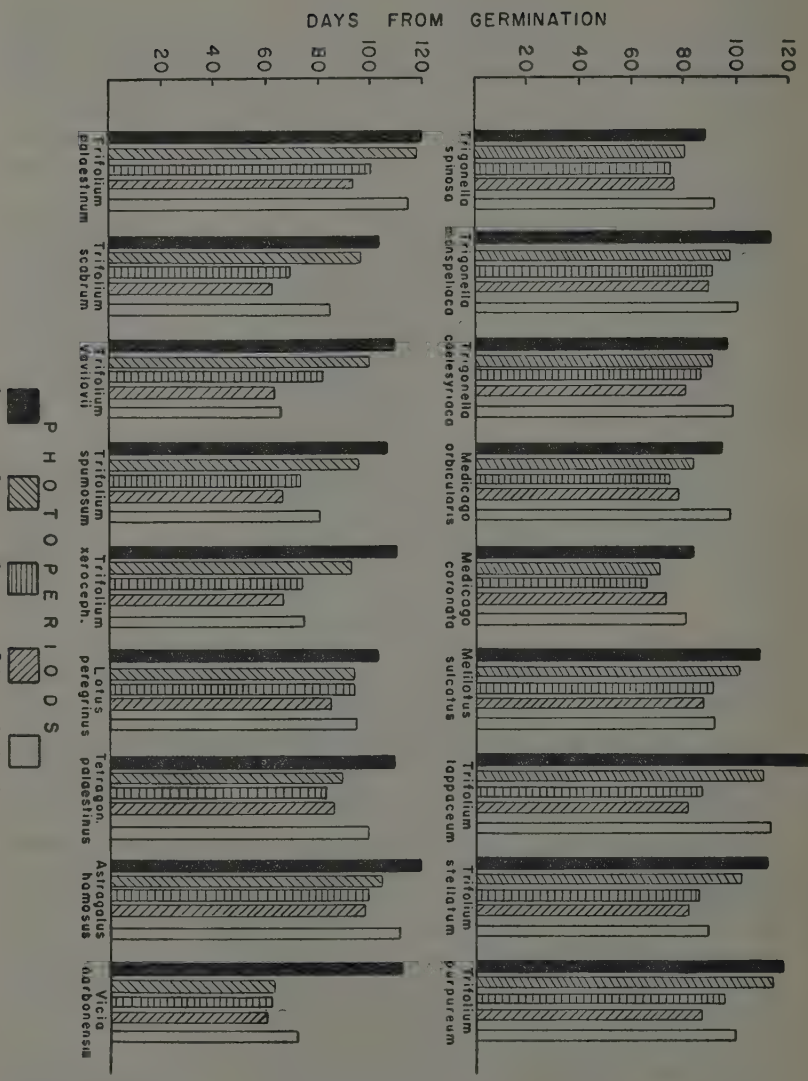

Figure 1
Effect of photoperiod on time of flowering.

RESULTS

Time of flowering

As examinations of primordia could not be carried out without injury to the experimental plants, the earliest opening of flower-buds was noted and taken as an indication of the onset of flowering. The day on which more than half of the plants to flower eventually reached this stage was written down as the date of flowering of a species under any given treatment. A histogrammatic representation of the number of days from germination to flowering is given in Figure 1.

The results of statistical analysis of the data represented in the diagram are summarized in Table I. Bracketed photoperiod indications form statistically homogeneous groups. Non-homogeneous groups differ significantly at $P = 0.05$.

TABLE I

Number of days from germination to the onset of flowering under different photoperiodic treatments

Species	Homogeneous groups	Estimates of means with confidence limits
Trigonella spinosa L.	(15,18,12,9, N*)	81.6±5.0
T. monspeliaca L.	(18,15); (12,N); (9)	89.5±7.9; 98.5±7.9; 113.0±11.1
T. coelesyriaca Boiss.	(18); (15,12,9); (N)	80.0±11.1; 90.7±6.4; 98.0±11.1
Medicago orbicularis (L.) Bartal.	(15,18); (12); (9,N)	75.5±7.9; 83.0±11.1; 95.5±7.9
M. coronata Desr.	(15); (12,18,N); (9)	65.0±11.1; 73.7±6.4; 83.0±11.1
Melilotus sulcatus Desf.	(18,15,N); (12,9)	89.3±6.43; 104.0±7.9
Trifolium lappaceum L.	(18,15); (12,N); (9)	82.5±7.9; 110.5±7.9; 128.0±11.1
T. stellatum L.	(18,15,N); (12,9)	83.7±6.4; 106.0±7.9
T. purpureum Loisel.	(18); (15,N,12,9)	85.0±11.1; 105.5±5.6
T. palaestinum Boiss.	(18,15); (N,12,9)	96.5±7.9; 116.7±6.4
T. scabrum L.	(18,15); (N,12); (9)	65.5±7.9; 90.0±7.9; 103.0±11.1
T. vavilovi Eig	(18,N); (15); (12,9)	64.0±7.9; 81.0±11.1; 104.0±7.9
T. spumosum L.	(18,15,N); (12,9)	73.0±6.4; 100.5±7.9
T. xerocephalum Fenzl.	(18,15,N); (12); (9)	71.0±6.4; 92.0±11.1; 109.0±11.1
Lotus peregrinus L.	(18,15,12,N,9)	93.2±5.0
Tetragonolobus palaestinus Boiss.	(15,18,12); (N); (9)	85.0±6.4; 98.0±11.1; 108.0±11.1
Astragalus hamosus L.	(18,15); (12); (N,9)	97.5±7.9; 103.0±11.1; 114.0±7.9
Vicia narbonensis L.	(18,15,12,N); (9)	63.2±5.6; 111.0±11.1

* Natural daylength.

In all the species tested, flowering was advanced with the increase of the photoperiod up to 15 hours. In a great majority of the species (including all representatives of *Trifolium*), the trend was maintained right up to the longest experimental photoperiod. Significantly delayed flowering under 18 hours as compared with 15 hours was found only in the case of *Medicago coronata*. In the confines of the daylength

extremes obtaining in Israel, there is no indication of a critical threshold and the flowering response to the length of day appears to be purely quantitative.

The behaviour of the plants under natural daylength merits special notice. It will be observed that natural daylength (which did not quite attain 12 hours in the greenhouse series) significantly advanced the flowering of *Melilotus sulcatus* and of some *Trifolium* species, as compared with the fixed 12-hour photoperiod.

The differences between the outdoor and indoor controls in the number of days from germination to the onset of flowering are taken as an indication of the effect of temperature on the time of flowering (Table II).

TABLE II

Effect of temperature on flowering at natural daylength

Species	Number of days from germination to flowering	
	High temp.	Low temp.
Trigonella spinosa	91	160
T. monspeliaca	100	153
T. coelesyriaca	98	154
Medicago orbicularis	97	158
M. coronata	79	140
Trifolium lappaceum	112	154
T. stellatum	88	170
T. purpureum	98	171
T. palaestinum	114	168
T. scabrum	84	170
T. vavilovi	65	156
T. spumosum	80	161
T. xerocephalum	74	158

L.s.d. for temperature effect at $P=0.01$: 50.54.
L.s.d. for temperature effect at $P=0.05$: 36.06.

It can be seen that in all the species tested flowering is appreciably advanced by high temperature.

Number of inflorescences

The total number of inflorescences produced by the experimental plants was recorded. In species producing single flowers, individual flowers were counted as inflorescences.

For the sake of simplicity, the pattern adopted in Table I is followed in Table III. Thus the values of individual treatment means are omitted; the means falling into statistically homogeneous groups are bracketed together in the order of increasing

TABLE III

Number of inflorescences under different photoperiodic treatments

Species	Homogeneous groups	Estimate of means with confidence limits
Trigonella spinosa	(18,12,9,N*); (15)	10.2±4.00; 24.0±7.99
T. monspeliaca	(N); (9,15,12); (18)	10.0±3.56; 13.2±2.06; 15.0±3.56
Medicago coronata	(18); (12,9,N); (15)	4.2±2.15; 6.6±1.24; 7.8±2.15
Melilotus sulcatus	(12,N,9,18); (15)	16.6±3.98; 34.6±7.97
Trifolium lappaceum	(9); (15,N,18); (12)	0.0±1.04; 1.4±0.60; 7.2±1.04
T. stellatum	(9); (12,N,15,18)	0.4±1.52; 2.2±0.76
T. scabrum	(9); (18,12,N,15)	3.8±1.52; 6.5±0.76
T. vavilovi	(12,15; (18)	3.4±2.07; 8.2±2.93
T. spumosum	(9,15,12); (18,N)	1.0±0.37; 2.3±0.45
T. xerocephalum	(9); (18,N,12); (15)	2.0±2.38; 4.1±1.38; 9.6±2.38
Lotus peregrinus	(15, 12,18); (N); (9)	5.9±1.23; 9.3±2.13; 12.3±2.13
Astragalus hamosus	(15,18,12,N); (9)	2.2±0.94; 4.7±1.87

* Natural daylength.

recorded values, and the estimate of the mean is supplied for each group. Only those species which yielded significant differences are included.

The effect of photoperiod on the *number of inflorescences* is much less pronounced than on the time of flowering, and is not nearly so consistent. In *Trifolium* there is an indication of suppressed production of inflorescences under short photoperiods. The optimal daylength varies with species and may be anything from a very short to a very long photoperiod. It is noteworthy that in the case of *Medicago coronata* a very long photoperiod, besides delaying the onset of flowering (see Table I), seems to suppress the formation of inflorescences.

Branching

The extent of branching has been indicated by the number of primary and secondary branches at the completion of flowering. It should be noted that in some of the species tested the plants remained green for a time after flowering, but growth increment was insignificant at this stage and in no instance was there any additional ramification.

Significant differences in the extent of branching due to photoperiodic treatment were established only for a few species and obvious trends were detected only within the genus *Trifolium*. While *T. stellatum*, *T. vavilovi* and *T. spumosum* responded to lengthening of the photoperiod with significantly increased ramification, an opposed trend was exhibited by *T. lappaceum* which produced a significantly smaller number of branches under 15 and 18 hours as compared with shorter photoperiods.

With the notable exception of *Tetragonolobus palaestinus* in which branching is significantly promoted by high temperature, ramification appears to be on the whole much more extensive in plants grown at low temperature (Table IV).

TABLE IV

Effect of temperature on branching at natural daylength

Species	Number of primary and secondary branches			
	High temp.	Low temp.	Difference	L.s.d. at $P=0.05$
Trigonella spinosa	1.8	4.4	2.6*	2.154
T. monspeliaca	0.0	1.8	1.8	2.154
T. coelesyriaca	0.0	6.3	6.3*	2.345
Medicago orbicularis	4.0	5.0	1.0	3.208
M. coronata	1.8	9.0	7.2*	2.688
Melilotus sulcatus	0.8	7.7	6.9*	2.388
Trifolium lappaceum	4.0	10.0	6.0*	2.026
T. purpureum	1.2	3.5	2.3	2.688
T. palaestinum	0.4	1.0	0.6	2.026
T. scabrum	1.5	4.8	3.3*	2.270
T. spumosum	1.6	2.0	0.4	2.154
T. xerocephalum	3.2	3.5	0.3	2.154
Lotus peregrinus	2.3	6.0	3.7*	2.623
Tetragonolobus palaestinus	4.0	1.0	3.0*	2.345
Astragalus hamosus	0.3	0.0	0.3	2.782

* Indicates significant difference.

Axial growth

Again, as in the case of branching, little consistency could be discerned in the effect of photoperiod on the total length of shoots. Greatest shoot elongation in *Medicago* and *Lotus* took place under the 9-hour photoperiod. Those species of *Trifolium* which produced significant differences varied strikingly in their response to the length of photoperiod. Thus maximum axial growth was associated with natural daylength in *T. stellatum*, with 12 hours in *T. lappaceum*, 15 hours in *T. xerocephalum*, 15 hours and natural daylength in *T. scabrum*, and 18 hours in *T. vavilovi*. It seems likely that, as far as vegetative growth is concerned, the response to photoperiod may be a cumulative function of a number of physiological components, such as direct morphogenetic effect, differences in the daily net assimilation rate and the duration of vegetative activity. The resulting pattern of photoperiodic response would thus reflect the relative ascendance of divergent trends in each species.

Temperature responses (Table V) show that, in analogy to ramification, with the exception of *Tetragonolobus palaestinus*, all the significant differences in the extent of shoot elongation are in favour of the lower temperature range.

DISCUSSION

The striking uniformity of the flowering response to daylength is rather surprising in view of the great variability of photoperiodic reaction reported for various legumes (Garner and Allard 1920, 1923; Maximov 1929; Allard and Garner 1940; Allard and Zaumeyer 1944), not infrequently for species and varieties of one and the same genus (Doroshenko and Rasumov 1929; Malinowski 1934; Hackbarth 1936). The quantitative response to the duration of photoperiod is equally interesting. This behaviour is hardly concordant with the traditional concept of long day plants, and it is felt that the more recent classification proposed by Chouard (1952) is more appropriate.

Thus most of the species would be best described as "*plantes héméropériodiques préférantes*" (or *quantitatives*). The genus *Medicago* is not sufficiently well represented to warrant generalization, but it seems that at least some of the species would be more fittingly defined as Chouard's "*plantes amphipériodiques*", viz. plants in

TABLE V

Effect of temperature on elongation of shoots at natural daylength

Species	Total length of shoots (mm)			
	High temp.	Low temp.	Difference	L.s.d. at $P=0.05$
Trigonella spinosa	191.5	405.6	214.1*	190.284
T. monspeliaca	167.5	307.2	139.7	190.284
T. coelesyriaca	199.2	599.3	400.1*	207.159
Medicago orbicularis	486.0	681.0	195.0	283.675
M. coronata	478.6	658.5	179.9	237.327
Melilotus sulcatus	218.0	1128.3	910.3*	216.651
Trifolium lappaceum	408.8	776.0	367.2*	179.319
T. purpureum	221.6	532.5	310.9*	258.959
T. palaestinum	152.2	264.4	112.2	179.319
T. scabrum	392.3	485.7	93.4	216.651
T. spumosum	166.6	103.3	63.3	190.284
T. xerocephalum	259.0	282.7	23.7	190.284
Lotus peregrinus	495.0	392.0	103.0	231.616
Tetragonolobus palaestinus	849.0	310.5	538.5*	258.958
Astragalus hamosus	137.3	152.0	14.7	245.666

* Indicates significant difference.

which flowering is delayed both by short and by very long photoperiods. The flower-promoting range being fairly wide, these medics could be more precisely classed as "plantes *eurypériodiques*".

As indicated by the results recorded for the natural daylength control, the gradual lengthening of the day may exert in some species a certain flower-promoting influence, distinct from the effect of fixed photoperiods. It is not unlikely that this may be due to the optimal photoperiod for flower initiation being shorter than that for flower development.

It is worthy of notice that the investigated species, nearly all of which come into flower in the early spring, in a region of a relatively short day, respond readily to such long photoperiods as 15 or even 18 hours, which can be assumed to be entirely outside the range of their evolutionary adaptations. It is unlikely that, in the absence of an operative critical threshold, changes in the length of day constitute by themselves the flower-regulating mechanism. The time of flowering appears rather to be determined by an interplay of photoperiod and temperature. The gradually increasing daylength and the rising temperature may be assumed to cooperate in the spring, until a combination of these two complementary factors favourable to flowering is reached.

It should be pointed out that the flowering response to temperature obtained in our experiments is rather different from that recorded for related pasture legumes (Roberts and Struckmeyer 1939, Ludwig et al. 1953) and actually indicates a behaviour diametrically opposed to that attributed to certain species of *Trifolium* and *Medicago* (Aitken 1955a,b). The outstanding uniformity of the temperature effect on the time of flowering in all the species tested suggests strongly that at least some of the species which served as parents for varieties of cultivated pasture legumes may also be 'high temperature plants'. The wide range of temperature requirements displayed by various types of *Trifolium subterraneum* (Aitken 1955a) points to considerable genetical variability as far as temperature responses are concerned, and there are indications in the other references cited that this may apply to other species as well. It is, of course, possible that many of the overseas selections are derived from Northern Mediterranean ecotypes with different temperature requirements. Bearing in mind the difficulty of securing flowering in some pasture legumes in the absence of low temperature induction (Aitken 1955a,b), one may look upon Israel legumes as a potential source of breeding material for hot climate pasture plants. Such breeding would obviously have to aim at combining readiness to flower with a prolonged growth period and abundant vegetative growth. It should be taken into account that, under the conditions of our experiments, the possibility of some vernalizing effects of outdoor temperatures during the germination and early seedling stage cannot be entirely dismissed; though this could have been, at most, very slight before mid-December. In any case, it is clear that while the response to the temperature level and to the photoperiod of the first few weeks after germination may be important (Aitken 1955a), it is by no means decisive.

As opposed to the strikingly consistent effect of photoperiod on the time of flowering, the lack of uniformity in the photoperiodic effects on the number of inflorescences is noteworthy. It will be noted, for instance, that the short 9-hour photoperiod, while suppressing the production of inflorescences in species of *Trifolium*, appears to exert a reversed effect in some other plants, such as *Lotus peregrinus* and *Astragalus hamosus*. The sequence of homogeneous groups in many of the species suggests that the effect of photoperiod on the formation of inflorescences may be con-

siderably modified by the secondary effect of the duration of the growth period. In plants with predominantly terminal inflorescences (e.g. *Trifolium*) photoperiodic effects on branching may be expected to be of considerable importance. In those species of *Trifolium* which showed significant effects there is indeed an indication of a marked correlation between the extent of ramification under the various photoperiodic treatments and the number of inflorescences.

In view of the absence of consistent trends in vegetative response to daylength, it seems likely that, beside any direct morphogenetic effects of photoperiod, the extent of growth at the close of the vegetative period may be promoted by increased net assimilation rate under long days, while the curtailment of the vegetative period is liable to impose a contrary tendency. Moreover, as far as axial growth is concerned, some degree of etiolation due to long hours of supplementary, relatively weak light may also affect the differential responses.

While the significant differences in vegetative development under the different temperature regimes may conceivably be brought about by morphogenetic effects, especially those of night temperature levels, the more extended vegetative period connected with delayed flowering is likely to constitute a factor of primary importance.

Our observations concerning the effect of environmental factors on vegetative development must be regarded as merely preliminary, since the experimental data refer only to arbitrary variables and their relevance is confined to a limited stage of development. A detailed investigation of selected species throughout the growth period is obviously needed to complete the picture.

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REFERENCES

1. Aitken, Y., 1955a, Flower initiation in pasture legumes; I. Factors affecting flower initiation in *Trifolium subterraneum* L., *Austr. J. agric. Res.*, **6**, 212.
2. Aitken, Y., 1955b, Flower initiation in pasture legumes; III. Flower initiation in *Medicago tribuloides* Desr. and other annual medics, *Ibid.*, **6**, 258.
3. Allard, H. A. and Garner, W. W., 1940, Further observations of the response of various species of plants to length of day, *Tech. Bull. U.S. Dep. Agric.*, **727.**
4. Allard, H. A. and Zaumeyer, W. J., 1944, Responses of beans (*Phaseolus*) and other legumes to length of day, *Tech. Bull. U.S. Dep. Agric.*, **867.**
5. Chouard, P., 1952, Le contrôle du photopériodisme dans la pratique horticole, *Rep. 13th int. hort. Congr.*, **2**, 820.
6. Doroshenko, A. V. and Rasumov, V. I., 1929, Photoperiodism of some cultivated forms in connection with their geographical origin, (Russian), *Bull. appl. Bot. Pl.-Breed.*, **22**(1), 219.
7. Duncan, D. B., 1955, Multiple range and multiple *F* tests, *Biometrics*, **11**, 1.

8. Garner, W. W. and Allard, H. A., 1920, Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants, *J. agric. Res.*, **18**, 553.
9. Garner, W. W. and Allard, H. A., 1923, Further studies in photoperiodism: the response of the plant to relative length of day and night, *Ibid.*, **23**, 871.
10. Hackbarth, J., 1936, Versuche ueber Photoperiodismus; III. Die photoperiodische Reaktionsweise einiger Lupinensorten, *Zuechter*, **8**, 81.
11. Ludwig, R. A., Barrales, H. G. and Steppler, H., 1953, Studies on the effect of light on the growth and development of red clover, *Canad. J. agric. Sci.*, **33**, 274.
12. Malinowski, E., 1934, Effect of the relative length of day and night on hybrid vigor in *Phaseolus vulgaris*, (Polish), *Roczn. Nauk rol.*, **33**, 50.
13. Maximov, N. A., 1929, Physiological factors controlling the length of the vegetative period, (Russian), *Bull. appl. Bot.-Pl. Breed.*, **20** (1), 169.
14. Roberts, R. H. and Struckmeyer, B. E., 1939, Further studies of the effects of temperature and other environmental factors upon the photoperiodic responses of plants, *J. agric. Res.*, **59**, 699.
15. Tukey, J. W., 1949, Comparing individual means in the analysis of variance, *Biometrics*, **5**, 99.

THE INFLUENCE OF DEPTH OF SOWING AND LIGHT CONDITIONS ON THE DEVELOPMENT OF THE PEANUT SEEDLING

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ABSTRACT

The response of peanut seedlings to variation in depth of sowing and light conditions was studied by size measurements. Seedlings were grown under various conditions of depth of sowing and of light, and measurements were made of their hypocotyls, epicotyls, total length of shoots, and roots. It was found that:

(1) The innate potentiality for hypocotyl lengthening in peanuts varies greatly. The maximum length obtained was 15 cm, but usually the hypocotyl length does not exceed 12 cm.

(2) Whenever the hypocotyl is unable to outgrow the depth of sowing, emergence is completed through further growth of the epicotyl and the rest of the shoot.

(3) Both the length of the hypocotyl and that of the underground section of the epicotyl tend to increase with the depth of sowing. The ratio between the two is irregular.

(4) The effect of light markedly decreases the lengthening of the hypocotyl.

(5) The cotyledons turn green when exposed to sunlight, but never develop into leaf-like form.

(6) The length of roots tends to decrease with increased depth of sowing.

INTRODUCTION

As stated by Hector (1936, p. 628), the germination of *Arachis* is epigeal. Gregory et al. (1951), however, state that the germination of the peanut is neither hypogeal nor epigeal, and that the lengthening of the hypocotyl is proportional to the depth of sowing, but cannot exceed the maximum length of 10—12 cm. Yarbrough (1949) obtained a hypocotyl length of 35—50 mm in peanut seedlings, when sowing depth was 5 mm, and suggested that the hypocotyl might grow longer with deeper sowing. Yarbrough also stated that peanut cotyledons may turn green if exposed to sunlight, in contradiction to Bouffil's (1947) finding.

Preliminary observations by the writer of this paper have shown that, although there is always considerable elongation of the hypocotyl in germination of peanuts, the cotyledons never appear above ground. The elongation of the hypocotyl seems always to stop just when the cotyledons reach the soil level. The experiments reported here were designed to determine the type of germination in peanuts, and to find how and to what extent the depth of sowing and light conditions influence the mode of germination.

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METHODS

Depth of sowing

Peanut seeds of the "Virginia Bunch" variety were sown in loose sand in a wooden box. Eight rows 8 cm apart were planted, each row containing 10 seeds with 3 cm spacing. The depth of sowing was increased by 2.5 cm for each successive row. Seeds in the first row were placed on the sand surface, while seeds in the last row were covered with 17.5 cm. Seeds were sown on June 19, 1955, and the seedlings were removed and measured on July 26, 1955. During this period the sand was kept moist.

Light conditions

A special container was prepared by removing two opposite sides of a wooden box and replacing them with glass panes. The container was filled with loose sand, and 7 seeds of the "Virginia Bunch" variety were placed in the sand next to each of the glass panes — one seed at each of the following depths: 0 cm, 2 cm, 5 cm, 8 cm, 11 cm, 15 cm, and 20 cm. To eliminate light at one end of the box, the glass pane was covered with black cloth firmly pressed against the pane. The other pane was left exposed. The sand was then dampened and kept moist for 4 weeks (May 4 to June 2, 1955), at which time the seedlings were removed, photographed and measured.

RESULTS

Depth of sowing

There was considerable variation in the amount of germination from the various depths. In Table I the extent of germination is presented, together with the means of measurements of the various parts of the seedlings. With each mean is also given (in bold face type) the variability coefficient of the data from which that mean was derived.

Table I shows a fairly steady increase in length of the hypocotyl with depth of sowing down to 10—12.5 cm. The variability coefficient is very high at depth 0, because the behaviour of the seeds germinating on the soil surface was most irregular. At the depth of 10 cm the variability coefficient is quite high, and at depths greater than 12.5 cm the regularity in the increase of hypocotyl lengthening is lost altogether. It can also be seen that emergence is not always achieved by the hypocotyl alone. At 5 cm and more in some seedlings the cotyledons were not pushed up to ground level, and emergence was completed through growth of the epicotyl. This contribution of the epicotyl to emergence tends to increase in length with increasing depth. On the other hand, there were cases, down to a depth of 12.5 cm, in which emergence was completed by the hypocotyl alone. Also, the ratio of the length of the hypocotyl to the length of the underground section of the epicotyl is most irregular. The total above-ground shoots are considerably shorter in seedlings from the greater sowing

depths. When the length of the above-ground shoot of each seedling was added to the length of the underground section of the epicotyl, it could be seen that the total length of that part of the seedling that developed above the cotyledons was not markedly affected by depth of sowing.

TABLE I

Means of measurements of various parts of peanut seedlings emerging from various depths

Sowing depth (cm)	Number of seedlings that developed out of 10 seeds	Hypocotyl length (cm)	Underground section of epicotyl (cm)	Total shoot above ground (cm)	Root length (cm)
0	5	1.24	0	26.6	16.4
		(81.5)	—	**(29.2)**	**(40.8)**
2.5	9	2.8	0	26.7	15.3
		(18.6)	—	**(18.4)**	**(15.7)**
5	10	5.3	1.0	25.0	12.5
		(15.5)	**(72.0)**	**(27.3)**	**(35.6)**
7.5	8	7.9	0.6	27.0	11.3
		(6.0)	**(121.1)**	**(13.0)**	**(5.8)**
10	8	9.4	2.6	20.8	7.1
		(33.1)	**(71.2)**	**(30.7)**	**(39.9)**
12.5	6	11.5	2.5	17.0	9.3
		(9.5)	**(85.7)**	**(17.4)**	**(33.0)**
15	7	8.9	7.0	12.6	9.0
		(22.1)	**(59.9)**	**(19.8)**	**(51.2)**
17.5	7	9.8	7.2	16.8	11.6
		(43.9)	**(34.7)**	**(12.4)**	**(52.7)**

Table I also shows that the root length tends to decrease with increased depth of sowing, down to 10 cm, and this is in accord with what is reported by Gregory (1951). It has been demonstrated by Bouffil (1947), however, that root-length varies inversely with planting depth to such an extent that peanuts germinating at their maximum depth for emergence are almost devoid of roots. In the present experiments greater root-lengths at the deeper sowing (Table I), which seem to contradict Bouffil's finding, are probably due to aeration through the bottom of the box — a condition which does not exist, of course, in the ground. Root measurements in the second experiment here reported (Table II) are more in accord with Bouffil's results (see also photographs).

Light conditions

An outstanding result of this experiment was the fact that under the influence of light, through the exposed pane, seeds at the greater depths produced seedlings with green leaves and no etiolation, well below the soil surface. On the side not exposed to light, seedling development was more like usual germination, though not quite like it, apparently due to the occasional influence of light at the very short periods (a few seconds only) when the pane was uncovered for observation.

Seedling measurements are presented in Table II.

TABLE II

The effect of light on the development of peanut seedlings at various depths of sowing

	Sowing depth (cm)	Hypocotyl length (cm)	Epicotyl length (cm)	Root length (cm)
A. exposed to light	0	0.6	1.0	18.5
	2	1.5	1.5	19.0
	5	1.0	1.0	16.5
	8	1.0	1.0	15.0
	11	1.5	1.5	14.5
	15	1.0	0.8	9.0
	20	1.5	1.5	4.5
B. not exposed	0	1.5	1.2	19.0
	2	—	—	—
	5	2.5	2.0	17.0
	8	3.0	3.0	16.0
	11	3.0	4.5	18.0
	15	3.5	3.0	3.0
	20	1.5	3.0	4.0

It can be seen from Table II that at all sowing depths, light caused shorter hypocotyls. The fact that in this experiment the hypocotyls at the greater sowing-depths were much shorter than at corresponding depths in the previous experiment, may be due to the occasional influence of light, mentioned above. This condition points to a possibility that the response of the hypocotyl to light is in some way similar to responses of photoperiodism, where very short breaks of a dark period are critical.

The cotyledons

It was observed in both experiments that wherever the cotyledons were exposed to light they turned green. They retained, however, their original form, though enlarged by swelling, and never developed into leaf-like organs, as the cotyledons of true epigeal legumes do. Observations of pre-emergence seedlings revealed that growth through the soil layers was not uniform: in some cases the cotyledons were pushed upwards in an upright position, piercing the soil with their tops; while in others the uppermost end of the hypocotyl was bent, to form an elbow-like projection, which headed the upward growth, and dragged the cotyledons with it. (See Figure 1.)

DISCUSSION

The results of both experiments point out that the peanut has a mode of germination, typical to itself, which can only slightly be modified by changing environmental conditions. This mode of germination cannot be classified either as hypogeal or as epigeal. In all cases the upward growth of the seedling starts with elongation of the hypocotyl; the extent of this elongation is determined by hereditary factors and by

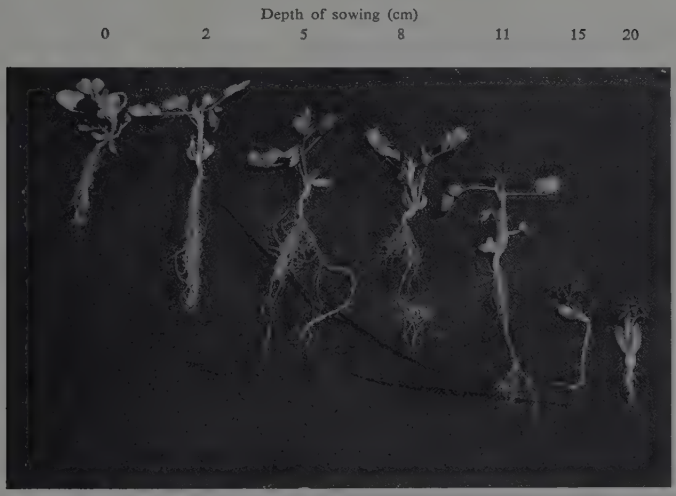

Figure 1
Peanut seedlings from seeds exposed to light, at various depths.

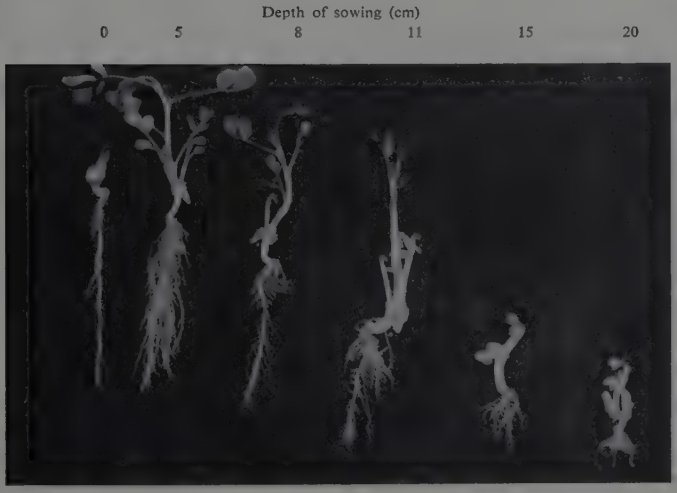

Figure 2
Peanut seedlings from seeds sown at various depths. Not exposed.

depth of sowing; it can also be influenced by light. The effect of light is apparently responsible for the arrest of hypocotyl elongation that occurs in peanut germination under field conditions, at the moment when the cotyledons reach the soil level. Although hypocotyl lengthening can be considerably reduced by the influence of light, yet it cannot be completely eliminated; even when the germinating seed is completely exposed to light and air, there is some lengthening of the hypocotyl. On the other hand, emergence of the seedling is not hampered if the hypocotyl is unable to outgrow the depth of sowing, but can be completed by further growth through the soil made by the epicotyl and the rest of the shoot. In this case, of course, the cotyledons remain underground.

Another interesting point concerning the mode of germination of peanuts is the fact that the combined length of the hypocotyl and the underground section of the epicotyl (or shoot) does not always equal the depth of sowing, i.e. the point of juncture of the hypocotyl and the root is not always found in the ground exactly where the seed had been placed, but either higher or lower than that. Such displacement could only be accounted for by assuming a possibility of some of the lengthening of the root being directed upwards, or some of the lengthening of the hypocotyl being directed downwards, as the case may be. On the whole, however, this point has not been studied sufficiently for making a clear statement on it.

The capacity of producing greatly varying combinations of hypocotyl and epicotyl length, and the particular position of the cotyledons during germination, certainly justify the placing of the peanut in a class of its own as regards type of germination.

REFERENCES

1. Bouffil, F., 1947, *Biologie, Ecologie et Selection de L'Arachide au Sénégal*, Ministère de la France D'Outre Mer.
2. Gregory, W. C., Smith, B. W. and Yarbrough, J. A., 1951, Morphology, Genetics and Breeding, in *The Peanut, The Unpredictable Legume. A Symposium.* The National Fertilizer Association, Washington.
3. Hector, J. M., 1936, *Introduction to the Botany of Field Crops. Non-Cereals*, Vol. 2, Centra News Agency Ltd., Johannesburg, S.A.
4. Nelson, Alexander, 1946, *Principles of Agricultural Botany*, Thomas Nelson and Sons Ltd., Edinburgh.
5. Yarbrough, J. A., 1949, *Arachis hypogea*, The seedling, its cotyledons, hypocotyl, and roots, *Amer. J. Bot.*, **36.**

OBSERVATIONS ON SOME *ACTINOMYCES* ISOLATED FROM SOIL SAMPLES FROM ISRAEL, AND THEIR MORPHOLOGY UNDER ELECTRONIC MICROSCOPY

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ABSTRACT

Eight strains of *Actinomyces* isolated from soil samples from the Northern Negev are described. The strains were referred to the following series: Albus, Rimosus, Viridis, Aureus, and Diastaticus.

The following general considerations about shape and morphogenesis of sporogenous hypha and spores in *Actinomyces* were suggested from observations of the cultures under the electron microscope:

(1) insertion of aerial hypha on a continuous tract of vegetative hypha;
(2) spiralization of sporigenous hypha when the segmentation has not yet occurred and the spores are not evident;
(3) segmentation of sporogenous hypha starting from the top towards the base;
(4) two patterns of spore release: (a) transverse fragmentation of sheath (exospore), (b) longitudinal split of same.

Prof. T. Rayss has invited us to study some strains of *Actinomyces* isolated from soil samples from the Northern Negev by S. Borut, to whose work (in preparation) the reader is referred for ecological characteristics and the technique of isolation. In this paper a study is presented of the biological characteristics of the isolated strains.

The first eight strains studied were isolated from the following soil samples:

P/1: loess soil from Mishmar Hanegev, at 10 cm depth.
P/2, P/3: sandy soil from just south of Beersheba, at 10 cm depth.
P/4: sandy soil from the plain of Treiba, at 50 cm depth.
P/5: sandy soil from just south of Beersheba, at 10 cm depth.
P/6: as P/5, at 25 cm depth.
P/7, P/8: as P/2, P/3, at 25 cm depth.

CULTURAL CHARACTERISTICS

All the strains, which came to us on potato-agar medium, were spread on the following media, at *p*H 7 to 7.2: potato-agar, Czapek agar, nutrient agar, asparagine-agar, potato plug, nutrient broth, starch-agar. For composition of the media see Baldacci, Spalla and Grein (1954).

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The morphological and cultural characteristics used here for series diagnosis (according to a classification studied at this Institute) concern the colour and appearance of the vegetative mycelium, colour of the underside of the cultures, colour and shape of the aerial mycelium, presence of diffusible pigments, and shape of the spores as observed under the electron microscope (Baldacci and Grein 1955).

To study these characteristics the strains were kept at a temperature of 30°C and observed on the third and tenth day. For observations on the thirtieth and fortieth day, the strains were kept at room temperature. For colour nomenclature Saccardo's Chromotaxia tables (1912) were used. Diastatic activity was investigated and antibiotic activity assays were performed. On the basis of morphological and cultural characteristics the strains were referred to the following series:

P/1: Albus series	P/5: Viridis series
P/2, P/3: Aureus series	P/6: Rimosus series
P/4: Diastaticus series	P/7, P/8: Diastaticus series.

P/1 strain, referred to the Albus series according to the characteristics of *Actinomyces albus* (Baldacci 1939), has a vegetative mycelium which is colourless on all mediums. (For this strain, as for every other, we considered the vegetative mycelium colourless when its appearance was similar to that of the medium.)

The underside of the culture appears constantly colourless; the aerial mycelium is scanty on potato plug and is straw coloured; the same colour and an even more limited growth are found on other media.

On the whole, the strain grows with difficulty in a thin rough patina; only on nutrient broth is the growth more abundant, appearing as a superficial veil which rather easily produces spores and becomes white. On starch-agar a halo of diastatic activity is found, about 3.5 cm in diameter. Under the electron microscope, the spores can be seen to have a cylindrical and sometimes rectangular shape, with eccentric connections (Figure 1). They therefore look slightly different from Albus

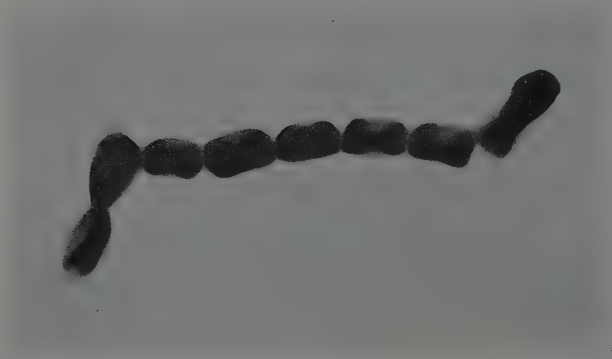


Figure 1
P/1 strain, Albus series, spores. (×10,000).

series, as described by Baldacci and Grein (1955), who observed oval and kidney-shaped, very often irregular, forms. The antibiotic activity assay showed a good inhibition of growth of *Staphylococcus aureus* and *Sarcina lutea*.

P/2 and P/3 strains show a vegetative mycelium which is at first amber coloured, and later egg yellow; while the underside shows an egg yellow tinge on every medium, except potato-agar, where the colour is closer to pale brick. The growth is good and the appearance is patinous. The aerial mycelium is white at the beginning, but later on most of the culture surface acquires a grey colour, giving the impression that the grey mycelium spreads over the white mycelium. On the whole it has a cotton-like appearance with many oozing little drops. The growth on broth is very scanty; even after 40 days few colonies can be found at the bottom of a tube. Under the electron microscope, the spores appear to be grouped in spirally rolled chains; they show an oval shape and very many thin long hairs (Figure 2). We were not able to state whether these features of the spores were characteristics of Aureus

Figure 2

P/2 strain, Aureus series. Chains of spores arranged in spirals. Note long thin hairs and segmentation of spores present only at the apex. (× 10,000).

series, because we have not so far examined any other strain of this series. The two strains have but a slight antibiotic activity on *Bacterium flexneri* and a very strong one on *Debariomyces marylandii*. They can be differentiated only by the diastatic activity, which appears to be much greater in P/3, the halo on starch-agar having a diameter of 6 cm against a 2 cm halo in P/2 strain. We can come to the conclusion that they belong to the same species, although likely to be different strains.

P/4 strain has a vegetative mycelium which is colourless on every medium; the underside of the culture is egg yellow coloured and the growth is very rich. The aerial mycelium is white at first, later on nearly totally grey. In nutrient broth it grows on the surface and produces spores abundantly. The diastatic activity halo on starch-agar is about 2.5 cm in diameter. The appearance of the spores in electron micrographs is typical of Diastaticus series (Baldacci, Grein and Spalla 1955). The strain is active on *Staphylococcus aureus* and *Sarcina lutea*.

P/5 strain has been referred to Viridis series (Baldacci 1939). The vegetative mycelium of this strain is colourless on every medium. The underside of the colony is amber coloured on asparagine-agar and light leather coloured on other media. The cotton-like aerial mycelium, with many oozing droplets, is white at the beginning and becomes eye blue later on. Only in broth does it give a brown pigment. It is noteworthy that the strains belonging to this series do not always pigment the agar medium and that the tinge of the aerial vegetation is variable from eye blue to green-

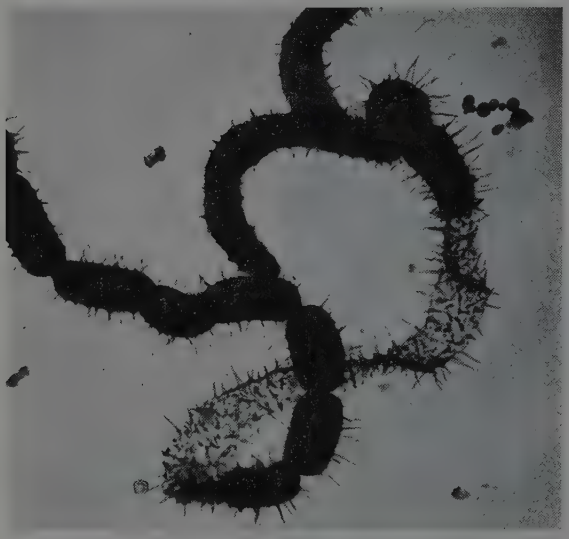


Figure 3
Chain of spores from P/5 strain, Viridis series. (× 10,000).

ish grey. On starch-agar it gives a diastatic halo about 3 cm in diameter. The spores, in spirally rolled chains, appear to be oval, non-transparent, endowed with long, spindle-shaped prickles (Figure 3). As shown in the photographs, the prickles belong to the sheath-like hyphal wall (exospore). In the same material, completely smooth spores can easily be seen (Figure 4); it is evident that we are dealing with naked spores shelled out of their exospore. But single spores do not always appear so smooth;

Figure 4
Smooth and prickly spores from P/5 strain, Viridis series. (× 15,000).

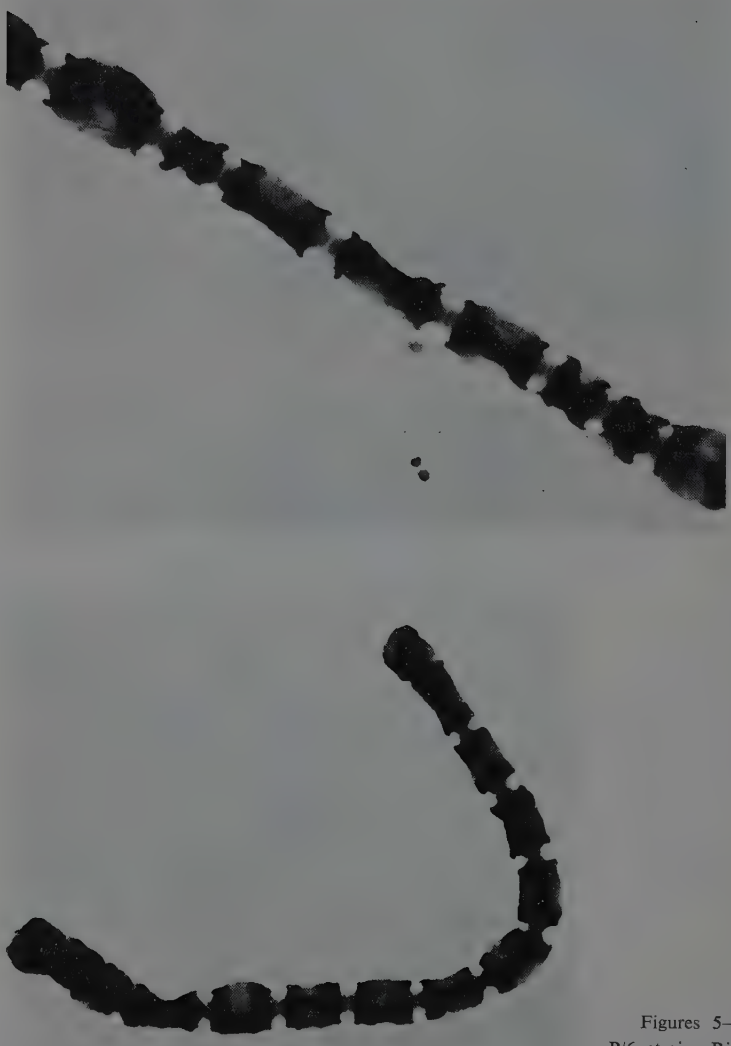
the general case is transverse splitting of the exospore and coating of single spores, which therefore retain the prickly appearance they show when grouped in chains. The strain is active on *Staphylococcus aureus* and *Sarcina lutea.*

P/6 strain has a vegetative date coloured mycelium on every medium and it grows abundantly. The underside of the culture is date coloured on potato-agar and nutrient agar, clearer on asparagine-agar. The aerial mycelium is grey and of a dusty appearance. It gives a brown pigment on asparagine-agar and on broth, where it grows downward. On starch-agar it shows a rather small halo of diastatic activity, 1 cm in diameter.

The spores observed under the electron microscope show a rectangular shape with four small horns on the corners. The opacity is not homogeneous and the connection between adjacent spores is rather unusual: every spore shows at its ends two cylindrical structures, adhering to the similar structures of the next spores. Between the two bulges a transparent septum can be seen. These structures are likely to belong to the exospore, and probably owing to their elastic consistency they were never seen either in isolated spores or in free ends of the chains (Figures 5, 6). This kind of connection has already been observed by Dreschler under the optical microscope in strains whose name is not given. In some cases the spores appear to be shrunken, perhaps following dehydration. Antibiotic activity is negative in the whole spectrum.

P/7 and P/8 strains are described together, as the only differences we were able to discern were that the growth pattern on potato plug was more abundant for P/7, and the spectrum of antibiotic acitvity varied.

The strains grow well on every medium, with a date coloured vegetative mycelium; underside of the culture from brown to deep date colour; grey, cotton-like aerial mycelium; strong pigmentation on nutritive agar, broth and potato plug. On starch-agar they give a halo of diastatic activity about 1 cm in diameter. The spores have



Figures 5—6
P/6 strain, Rimosus series; chain of spores. Note structure of insertion points. (×10,000 — 12,000).

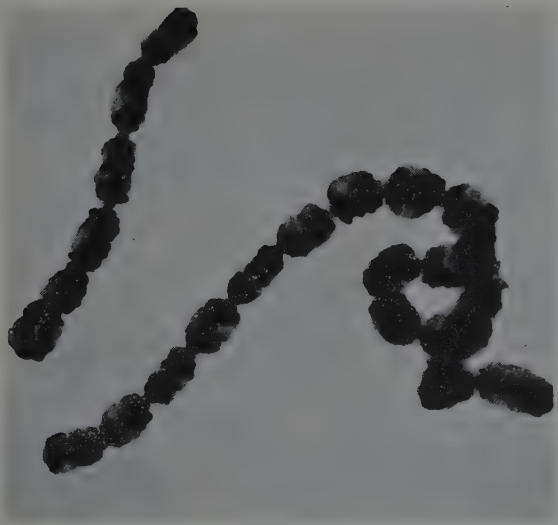


Figure 7
P/8 strain, Diastaticus series; bumpy spores. (× 10,000).

a shape similar to that of Diastaticus series; they show a number of bulges and optically they are markedly non-homogeneous.

Their connection points are not so different from those described for the spores of the P/6 strain (Figure 7).

From the point of view of antibiotic activity, while P/7 strain is negative in the whole spectrum, P/8 strain shows some effectiveness on *Staphylococcus aureus* and *Sarcina lutea*.

MORPHOLOGY

The strains which have been isolated can all be included in the series already described. Nevertheless, we think it of interest to note that some of them produce spores of a shape which has not been described so far. For instance, filaments as long as those belonging to the spores of P/2 and P/3 strains are, as far as we know, completely new features. The morphological observation of the strains presented in this paper suggests a few general considerations about shape and morphogenesis of the sporogenous hypha and the spores in *Actinomyces*.

Figure 8 performed on P/7 strain, shows clearly the insertion point of the aerial hypha on the substratal mycelium. The connection shows an angle of about 90° and lies on a continuous tract of the vegetative hypha.

This pattern of insertion has been noted previously by Carvajal (1946) in *S. griseus* and by us on many strains, and therefore we think that this is the usual origin of the sporogenous hypha. On the other hand, we have never been able to see any features that could be referred to the so-called "initial cells" (Klieneberger-Nobel 1947),

Figure 8
P/7 strain, Diastaticus series; immature aerial hypha. Note optical density, higher than that of the vegetative hypha, spiralization, beginning of segmentation and origin of aerial hypha on a continuous tract of the vegetative mycelium. (× 4,500).

crossing points of two vegetative hyphae, from which the aerial hyphae would take their origin; but we cannot exclude this insertion pattern. On the same material we can see that the optical density of the sporogenous hypha appears to be higher than that of the vegetative hypha, and that the spiralization of the former is present when the segmentation of the hypha has not yet occurred and the spores are not yet evident. As for the debated question, whether the spores differentiate first at the base or at the top of the hypha (or simultaneously), Figure 2 shows that the segmentation starts at the top and continues along the aerial hypha towards the base.

Shape and dimensions of spores are not always the same and the differences become greater as the culture grows older. To a certain extent these differences are due to various degrees of dehydration of the spores, to a greater extent to the fact that along the chain, above all in cases of spiralized hyphae, the spores, nearly always flattened, show sometimes the larger, sometimes the narrower face, being variously arranged round the axis of the hypha (Figure 9). Technical artefacts are also likely to play an important part in this connection. In fact, the action of the electronic pencil on all the examined materials and above all on newly formed spores is not without effect. Chains of spores under an electronic pencil of the intensity required for greater magnifications become longer, their narrowing points become less distinct, they very often look much like spindles (cf. Figure 10 with Figure 1).

Figure 9
Two pictures from the same chain of spores, before and after rotation of 90°, to show the flattened shape of the spores. Note especially the second and third spores from the tip. (× 8,000).

These points must be stressed for every description of spores under the electron microscope. We have already mentioned how single spores can be set free from the chains. This can happen either through a transverse fragmentation of the sheath, or through a longitudinal split. In the former case the exospore is still adherent to the isolated spores and, in the case of prickly or hairy aerial hypha, they will maintain their characteristic appendices. In the latter case the exospore appears to become empty and the outline of the spores is always smooth. This second event has already been referred to by Vernon (1955), but he did not mention the other alternative. From our point of view we feel bound to advance the hypothesis that the longitudinal split may be due to an artefact of the electron microscopy technique, also considering that this pattern of splitting, as far as we know, has not been described by any author who made his observations under an optical microscope.

That in *Actinomyces* a haploid and a diploid phase exist, seems to be demonstrated by the work of Sermonti and Spada Sermonti (1955). He crossbred two strains with different nutritional deficiencies and obtained prototrophic recombinants and recombinants carriers of deficiencies coming from both parents. It is most likely that the diploid phase in the life-cycle is very short; but it is rather difficult at the present moment to state how short it is and to which morphological structure, among those that have been described, the diploid phase corresponds (Jones 1955). The work of Erikson (1948), who isolated sister spores from the same chain obtaining dissimilar growths, suggests the hypothesis that conidia are diploid and that meiosis takes place within the spore, the nucleus of the germinal tube being haploid as a product of this meiosis.

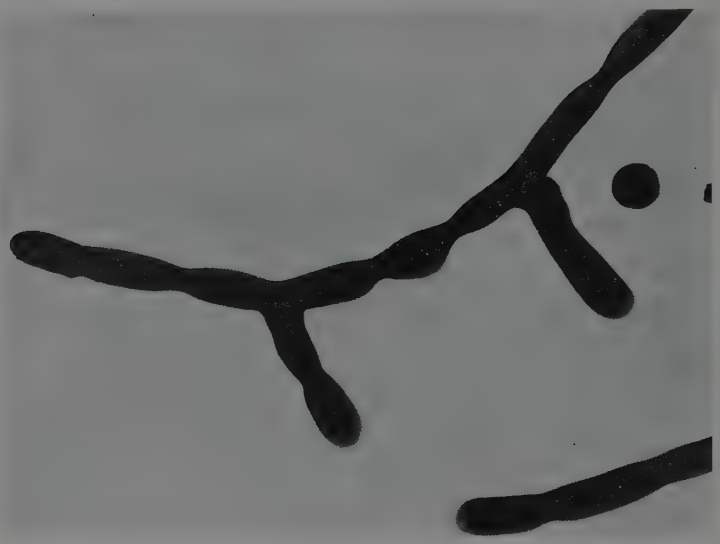


Figure 10

P/1 strain; chain of spores after prolonged exposure to the electronic pencil. (× 10,000).

REFERENCES

1. Baldacci, E., 1939, Contributo alla sistematica degli Attinomiceti, *Att. Ist. bot. Univ. Pavia* V—VIII Series IV, **11**, 192—231.
2. Baldacci, E., Comaschi, G. F., Scotti, T. and Spalla, C., 1953, General criteria for the systematics of genera and species of *Actinomyces* (*Streptomyces*) and Micromonospora, Symposium Actinomycetales, *Suppl. Rend. Ist. sup. Sanit.*, 20—39.
3. Baldacci, E. and Grein, A., 1955, Esame della forma delle spore di Attinomiceti al microscopio elettronico e loro valutazione ai fini di una classificazione, *G. Microbiol.*, **1**, 28—34.
4. Baldacci, E., Grein, A. and Spalla, C., 1955, Studio di una serie di specie di Attinomiceti. A. Diastaticus, *G. Microbiol.*, **2**, 127—143.
5. Baldacci, E., Spalla, C. and Grein, A., 1954, The classification of the Actinomyces species (Streptomyces), *Arch. Mikrobiol.*, **20**, 357.
6. Borut, S., Soil fungi of the Northern Negev, in preparation.
7. Carvajal, F., 1946, Studies on the structure of *Streptomyces griseus*, *Mycologia*, **38** (5), 587—595.
8. Erikson, D., 1948, Differentiation of the vegetative and sporogenous phases of the Actinomycetes. 3. Variation in the *Actinomyces coelicolor* species group, *J. gen. Microbiol.*, **2** (3).
9. Jones, K. L., 1955, Variation in Streptomyces, *Ann. N.Y. Acad. Sci.*, **60.**
10. Klieneberger-Nobel, E., 1947, The life cycle of sporing Actinomycetes as revealed by a study of their structure and septation, *J. gen. Microbiol.*, **1**, 22—37.
11. Saccardo, P. A., 1912, *Chromotaxia seu nomenclator colorum*, Patavii Typis Seminarii.
12. Sermonti, G. and Spada Sermonti, I., 1955, Genetic recombination in Streptomyces, *Nature, Lond.*, **176**, 121.
13. Vernon, T. R., 1955, Spore formationin the genus *Streptomyces*, *Nature, Lond.*, **176**, 935.

EFFECT OF LIGHT ON SPORULATION OF *TRICHODERMA VIRIDE* PERS. EX FRIES *,**

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ABSTRACT

Trichoderma viride Pers. ex Fries belongs to those fungi, the sporulation of which is strongly stimulated by exposure to light. Growth in continuous darkness results in a failure or marked delay of spore formation, and causes changes in cultural characteristics. Where cultures are transferred from light to darkness, the effect on sporulation seems to some extent "retroactive": the diameter of the spore-bearing central disc is therefore smaller than the diameter of the colony at the moment of transfer. A similar "retroactive" effect appears, when cultures are transferred from darkness to light.

Alternating light and darkness induce formation of alternating concentric rings of spores and spore-less mycelium. With some isolates, the width of each consecutive ring increases while the density of spores decreases. A single exposure to light results in the formation of one ring of spores only.

The effect of length of exposure to light on subsequent sporulation was studied. Exposures of 20—60 seconds to light of 85—90 lux intensity suffice to induce some sporulation.

The effect of light intensity in conjunction with various lengths of exposure was also studied. A reciprocal time-intensity relationship, conforming with the Bunsen-Roscoe law, was found.

Light induced sporulation only when mycelium was exposed to it at a definite stage of growth. No such effect was obtained by exposure of spores at various stages of germination, of old mycelium, or of mycelium in which scarce development of spores is already taking place.

Preliminary experiments indicate that presence of oxygen is necessary to permit light to induce spore formation.

INTRODUCTION

Light is very often known to have a striking influence on many processes in the life-history of the fungus, such as: vegetative growth, sporulation, discharge of spores and their germination, pigmentation, enzymatic activity, etc.

Many observations have been published on the effect of light on spore formation (Bisby 1925, Houston and Oswald 1946, Hawker 1950, Lilly and Barnett 1951, Miller, Webb and Reid 1953, etc.), their number, size (Harter 1939) and septation (Harter 1941). Generally, an exposure to light results in a stimulation of sporulation,

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Figure 3

Effect of light and of darkness on sporulation of five isolates of *Trichoderma*. — Left to right: isolates No.: 12, 10, 11, 8, 13. Upper row: continuous light. Middle row: alternating light and darkness. Lower row: continuous darkness.

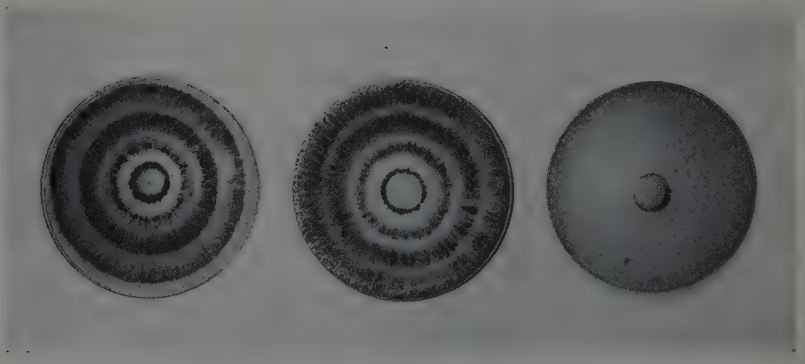

Figure 4

Concentric rings of spores formed under the influence of one-hour exposures given once every 24 hours. (Petri-dishes 23 cm diameter). Left: isolate No. 8. Right: isolate No. 10.

Figure 5

Effect of a single one-hour exposure on sporulation of isolate No. 8 of *Trichoderma*. (Petri-dish 23 cm diameter).

but in some fungi the light inhibits, or at least delays, the formation of spores (Hawker 1950). Thus, the reaction of the fungi to light exposure is not unique, and Hawker (1950) divides them into 4 groups according to the influence light has on them.

In spite of the great number of observations on the effect of the light on micro-organisms, there are only few reports which give data regarding the intensity and quality of light studied, length of exposure tested and other details.

Trichoderma viride Pers. ex Fries belongs to those fungi, the sporulation of which is strongly stimulated by exposure to light (Lilly and Barnett 1951, Barnett 1955, Miller 1955). The information, however, regarding the effect of light on sporulation of *Trichoderma* was rather scarce.

The study of the process of spore formation is of both theoretical and practical importance. Experiments were, therefore, carried out to study the effect on sporulation of *Trichoderma* of such factors as: light and darkness, length of exposure, intensity of light, stage of development of fungus, presence of oxygen and others.

MATERIALS AND METHODS

All experiments were carried out on five different isolates of *Trichoderma*, Nos. 8 and 10 (in our collection) isolated from the crown of a sweet-lime rootstock, No. 12 from rotted textile, No. 13 from dung (isolated in Israel), and No. 11 isolated from soil in England*. These were grown on 2% potato-dextrose agar in Petri dishes. Spore suspensions in distilled water served as inoculum. The cultures were kept at a room temperature of 26°±2°C — a range which falls within the optimum for growth of this fungus. The experiments were carried out in triplicate.

Forty-watt daylight fluorescent lamps, the spectrum of which is almost identical with that of natural daylight (Paterson 1946), served as source of light. The cultures were kept at a distance of 2 m, and the intensity of light reaching the fungus was 85—95 lux, as measured with a selenium photo-electric cell. (Throughout the "life" of a fluorescent lamp, a decrease of less than 10% in the intensity of light was measured).

Cultures to be kept in darkness were given the same conditions of temperature and humidity, but were wrapped in black, non-transparent paper and shielded from light.

When observations of cultures during the "dark period" were to be made, the following alternative methods were employed: either the cultures were examined briefly under a weak, indirect red light, or the number of replicates was increased at the beginning of the experiment and groups of cultures were later, at required intervals, withdrawn from darkness and transferred to light for observation.

Cultures kept under continuous light and darkness served as controls.

EFFECT OF LIGHT AND OF DARKNESS ON SPORULATION

Continuous light

Five isolates of *Trichoderma* were grown in continuous light. Observations on the effect of light on their sporulation are given in Table I.

* Thanks are due to Dr. I. Levisohn, Bedford College, London, and Prof. T. Rayss, The Hebrew University of Jerusalem, for providing isolates Nos. 11 and 12 .

Continuous darkness

The identical five isolates were grown in continuous darkness and examined from time to time during the experiment (as described in the previous chapter) (Table I).

TABLE I

The effect of continuous light or darkness on sporulation of five isolates of Trichoderma

Grown in	Isolate No. 8	10	11	12	13
Continuous light	within 4—5 days entire plate covered by spores homogeneously distributed	as No. 8	within 6—7 days periphery of plate covered by tufts with spores	within 5—6 days entire plate covered by spores formed radially (no tufts)	as No. 8 (5 days)
Continuous darkness	after 8—10 days few tufts with spores begin to form at centre of colony. Remainder of plate free of spores	after 6—10 days few spores begin to form, mostly at centre of colony	after 10—12 days several tufts with few spores begin to form at periphery of plate	after 7—10 days few yellowish-green tufts begin to form at periphery of plate	as No. 8

The observations made have shown that the growth in continuous darkness causes several changes in the culture, as follows:

(a) *Retardation of spore formation*

Amount of spores. No spores or only a very small amount of spores were formed in cultures grown in continuous darkness (Figure 1B), as compared with the profuse spore formation in light (Figure 1A).

Delay in spore formation. The sporulation in cultures grown in continuous darkness — if spores formed at all — was delayed by several days up to one week, as compared with cultures grown in continuous light.

(b) *Growth habit of the fungus colony*

Sometimes (e.g. isolate No. 12) tufts with spores are formed in culture grown in continuous darkness, whereas in light the same culture forms spores evenly distributed over the whole plate.

In darkness spores are very often formed at the centre of the colony or at its periphery only, while in continuous light the whole plate is covered by spores.

(c) *Colour*

The deep-green or bluish-green colour of the culture, grown in continuous light, changes to yellowish-green or light-green, if the same culture is grown in darkness.

Observations have also shown that there was no difference in rate of growth of mycelium between cultures grown in darkness and those grown in light.

It should also be mentioned that though some of the plates, grown in continuous darkness, were observed under red light, no perceptible changes were found at the end of the experiment between plates grown in darkness and those exposed for short periods to red light.

Growth in light followed by transfer to darkness

The five isolates mentioned above were grown in light. 54 hours after inoculation, when the diameter of the culture reached 40 mm, the plates were transferred to darkness until the end of the experiment. All cultures formed a "disc" of spores at the centre of the colony. The diameter of this "disc" in all isolates, but No. 12, was smaller by 10 mm than the diameter of the colony, at the moment of transfer from light to darkness. The remainder of the plate outside this "disc" was free of spores (Figure 1C).

With isolate No. 12 a similar "disc" of spores was formed, but its diameter was equal to that of the colony at transfer from light to darkness. There was only very scarce formation of spores outside this "disc".

As mentioned above, the diameter of the "disc" of spores, was — with the exception of isolate No. 12 — smaller than that of the colony at the moment the exposure to light was discontinued (Figure 1C). This means that the youngest mycelium did not form spores. At first it was thought that the amount of light this mycelium received was insufficient to stimulate spore formation by the irradiated mycelium. Later experiments, however, proved this to be untrue, and therefore it is assumed that the mycelium formed shortly before cessation of irradiation is too young — or the required differentiation had not yet taken place — and is unable to respond to the stimulus of light by forming spores.

Darkness followed by light

In this experiment the cultures were kept in darkness for 54 hours after inoculation and were then transferred to light. A reverse picture was obtained: at the centre of the colony there was a region free of spores, while the remainder of the plate was covered by spores. The diameter of the spore-less region was smaller by 13—20 mm than that of the colony at transfer to light (Figure 1D). This shows that the light induced spore formation in the mycelium formed before the beginning of the exposure, but only in outer part of this mycelium, and thus a spore-less region still remained at the centre. It seems, therefore, that if the time elapsed between the formation of mycelium in darkness and exposure to light is too long, the mycelium loses its ability to react to the stimulating effect of light.

With isolate No. 12, the diameters of both mycelial growth and spore "disc" are equal and there is no "shift" towards the centre of the colony. This immediate

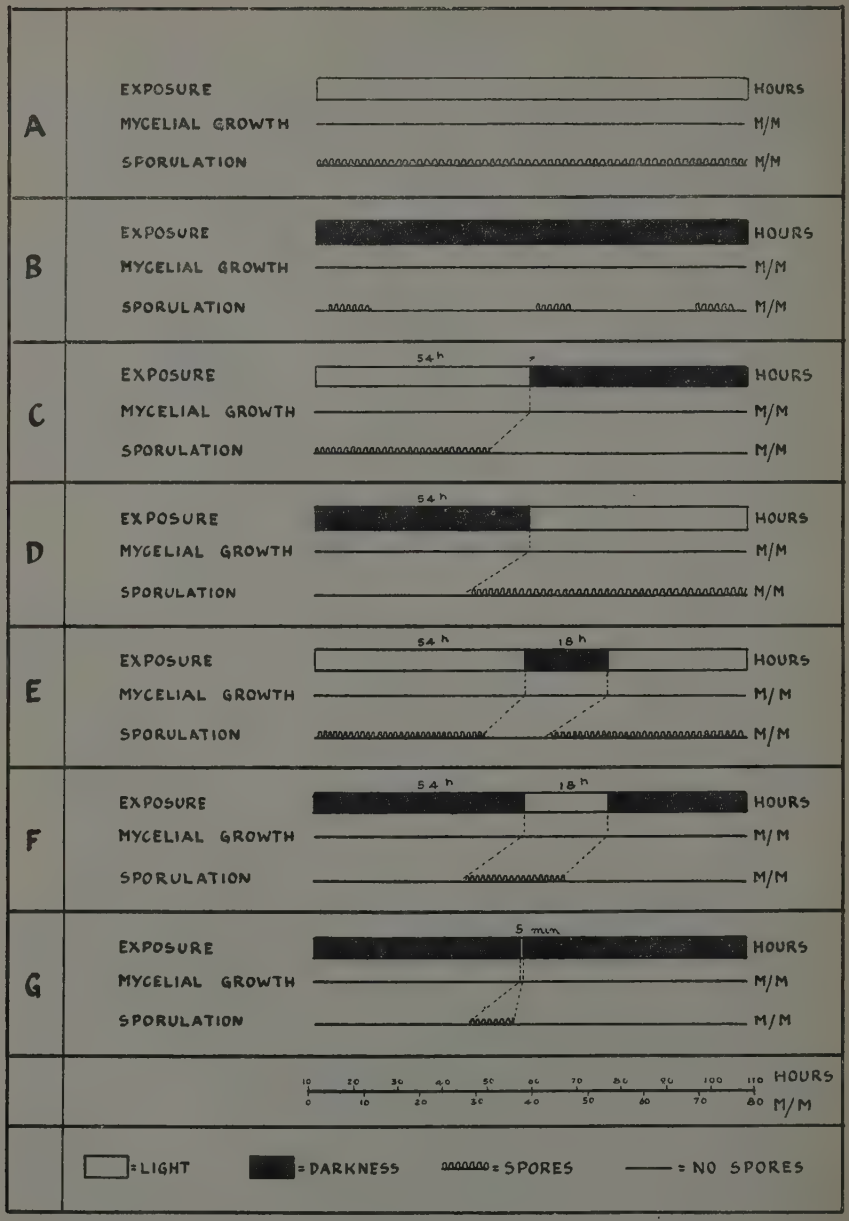

Figure 1
Effect of light and of darkness on sporulation of *Trichoderma*.

reaction to the exposure to light or its cessation seems to be a feature characteristic to this isolate, since it was repeated in all the other experiments (Figure 2).

It should also be mentioned that prolonged exposure to light at the end of the experiment did not alter the picture obtained. This holds true for all isolates and also to all other experiments to be described later.

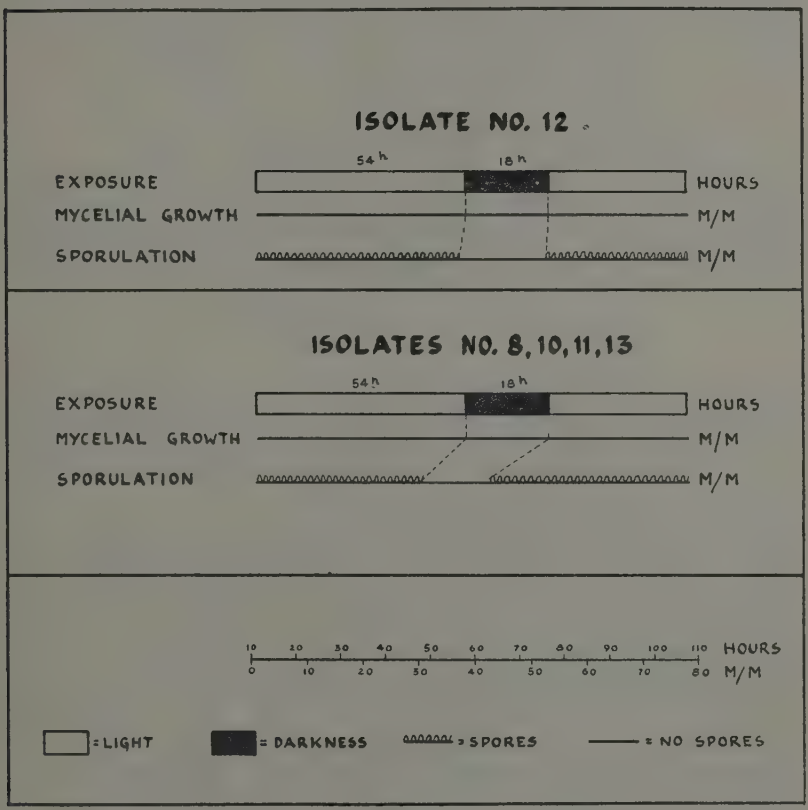

Figure 2

Differences between various isolates of *Trichoderma* in their reaction to light.

From this and the previous chapter it may be concluded that only a mycelium of suitable age is able to react to the stimulating effect of light: light does not seem to induce spore formation either on too young a mycelium, formed just before the exposure, or on too old a mycelium.

Continuous light interrupted by a period of darkness

The cultures were grown in light for 54 hours after inoculation, transferred to darkness for 18 hours, and then returned to continuous light.

The result was that the whole plate was covered by spores, except for a 10—15 mm wide spore-less ring. Thus, three zones were obtained: spores—mycelium—spores, which corresponded to the periods of light—darkness—light to which the fungus was exposed. It should be stressed that the spore-less ring has undergone a "shift" towards the centre of the culture as compared with the diameters of the colony at the moments of transfer (first to darkness and then back to light) (Figure 1E).

Continuous darkness with one exposure to light

In this experiment the order was reversed: cultures were grown in continuous darkness except for one 18-hour-long exposure to light. As could be predicted, a dense ring of spores was formed, with spore-less mycelium on both its sides. The peculiar "shifting" of the ring towards the centre of the colony was again observed here (Figure 1F).

EFFECT OF ALTERNATING LIGHT AND DARKNESS

It may be assumed that the first observation to the effect that light induces spore formation in *Trichoderma* was made during the growth of this fungus under laboratory conditions: alternating concentric rings of spores and spore-less mycelium were formed under the alternating influence of daylight and darkness of the night (Figure 3).

To study the effect of alternating light and darkness, an experiment was carried out, in which cultures of isolates Nos. 8, 10 and 12 were grown in continuous darkness with one-hour exposures given to them once every 24 hours. This was compared with another group of plates to which only a single 1-hour exposure was given, as well as with controls, in which same isolates were grown under conditions of continuous light or darkness. Large Petri dishes (14 cm and 23 cm in diameter) were used to prolong the observation period, increase the growth area, and enable the repeated formation of concentric rings of spores.

As expected, every exposure brought about the formation of a ring of spores. But it was found with isolates Nos. 8 and 10 that the width of each consecutive ring of spores increased, while the density of spores decreased (Figure 4), in spite of the fact that all exposures were for one-hour only and given at equal intervals. However, the width of the rings of spores formed by isolate no. 12, and the distances between them, were constant.

Cultures given a single exposure (44 hours after inoculation) formed one ring of spores only and, with the exception of few tufts near the rim of the plate, no spores were found on its remaining part (Figure 5). Hence, one exposure is unable to stimulate the formation of more than one ring of spores.

In the controls, in continuous light, the whole plate was covered by spores, while in continuous darkness, except for a few tufts near the rim of the plate, no spores were formed.

LENGTH OF EXPOSURE

The following experiments aimed at studying the effects of the length of the period of exposure and determining the shortest exposure required for a perceptible stimulation of spore formation.

(1) Exposures of less than 5 minutes were first studied. The cultures were grown in complete darkness and the single exposure from a source of light of 85—90 lux intensity was given 60 hours after the inoculation. The periods of exposure tested were: 2, 5, 20, 60 seconds and 5 minutes. Cultures grown in continuous light or darkness served as controls.

Results have shown that 2 and 5 seconds' exposures had no perceptible effect on the cultures, which grew as in continuous darkness. An exposure of 20 seconds given to isolate No. 12 resulted in the formation of a distinct ring of spores, while with the other (three) cultures a minimum exposure of 60 seconds was required. With the increase of the length of exposure to 5 minutes, the ring became still more distinct (Figure 1G).

Increasing in all isolates the length of exposure up to 1 minute, induced an increase in the density of spores in the ring and a clearer demarcation of the ring; absence of the spores anywhere but in the ring itself was complete only after the five minutes' exposure.

These observations show that an exposure of 20—60 seconds suffices to demonstrate the stimulating effect of light, and that light may alter cultural characteristics of the fungus and bring out differences between isolates, which would otherwise escape notice.

(2) In another experiment periods of exposure exceeding 5 minutes were studied. After 60 hours of incubation, cultures of isolate No. 10 were given the following exposures: 5 and 30 minutes, 2, 6, 12 and 24 hours. Before and after the exposures the cultures were kept in darkness.

It was found that the exposures from 5 minutes to 6 hours brought about the formation of a ring of spores, without any spores anywhere outside that ring. After a 24 hours' exposure, the ring was not well defined, the density of spores smaller and the whole appearance resembled that obtained after exposure to continuous light. The results of 12 hours' exposure showed a transition from those of the shorter to those of the longer exposures.

INTENSITY OF LIGHT

To study the effects of the intensity of light, fluorescent lamps of identical spectrum, but different intensity, were used. Two intensities: 85—90 and 650—700 lux, and five

exposure times: 3, 10, 30, 90 seconds and 5 minutes, were tested on cultures of isolate No. 8, which were kept in darkness before and after exposure.

The results showed that the same effect was obtained by 30 seconds' exposure to light of 90 lux intensity as by 3 seconds' exposure to 700 lux. Similar relationships were obtained with other levels of light intensity and different periods of exposure. It may, therefore, be assumed that the effect of exposure to light is related to the amount of light, which depends on the intensity of the source of light and the length of exposure: a short exposure to light of high intensity has an effect similar to that of prolonged exposure to weak light.

Thus, to stimulate spore formation, a given constant amount of light appears to be required. Any additional exposure exceeding this "threshold value" does not result in any perceptible change in the culture.

It seems that additional experiments may result in finding a mathematical correlation between the intensity of light and the length of exposure on one side and the results of the exposure on the other, and also contribute to a better understanding of the mechanisms of sporulation, as induced by light.

EFFECT OF EXPOSURES GIVEN TO MYCELIUM AT VARIOUS AGES

Experiments were carried out in which cultures of isolates Nos. 8 and 10 were grown for different periods (from $1\frac{1}{2}$ to 15 days) in darkness, and then either given a 6 hours' exposure and returned to darkness, or transferred to continuous light.

It was found that light still induced spore-formation on a mycelium grown in darkness for not more than 5 days. But the same exposure given to a culture grown for 7 or more days in darkness, had no such effect.

Sometimes, as shown in Table I, very weak and scattered sporulation occurred after 6 or more days in cultures kept in darkness. The amount of spores could not be increased by subsequent exposure to light, no matter how long it lasted.

EFFECT OF EXPOSURES GIVEN TO SPORES AT VARIOUS STAGES OF GERMINATION ON SUBSEQUENT SPORULATION

During this work, the question arose, on which stage of the development of the fungus light has a sporulation-inducing effect, and whether an exposure given to the fungus at one stage of its growth may induce sporulation at another.

Petri dishes with potato-dextrose agar were seeded with a spore suspension of isolate No. 8, and a 5-minutes' exposure to a light of 700 lux was given after the following periods: 4 hours after seeding, the approximate time required by the spores to start swelling, 12 hours, corresponding to the beginning of germination, and 24 hours, when germination was advanced and germ-tubes formed. Before and after the exposure the cultures were kept in darkness. Cultures not exposed to light served as controls.

Results have shown that there was no difference between the exposed cultures and the controls. It seems, therefore, that an exposure to light given to the spores

of *Trichoderma* at any stage of germination does not induce subsequent sporulation in the mycelium to which they give rise.

It may be assumed from this as well as from previous experiments, that the light stimulates sporulation at a given stage of the growth of a mature mycelium only; it has no effect either on spores at the various stages of their germination, or on an old mycelium or a mycelium in which a scarce formation of spores has already taken place.

EFFECT OF LIGHT AS INFLUENCED BY PRESENCE OF OXYGEN

It may be suggested that the above described stimulation of sporulation in *Trichoderma* as affected by light, is connected with some oxidation processes. A similar suggestion was already made by Coons (cited from Leonian 1924).

To study this aspect, cultures of the fungus grown in light and darkness, respectively, were kept in the presence or absence of oxygen. Cultures (isolates Nos. 8 and 10) in Petri dishes were put into 3.5 litre jars, to each of which 100 cc of 20% KOH and 30 cc of 44% pyrogallol were added. The absorption of oxygen was accomplished by bringing the two solutions into contact, after the jar with the cultures had been hermetically sealed. The degree of absorption was shown by the oxygen indicator (methylene blue in its leuco form).

Where cultures 48 hours old were kept for 2 hours without oxygen, a spore-less ring was formed. No spores were formed on this ring even after oxygen was restored.

The effect of prolonged lack of oxygen was studied in cultures which after 48 hours of incubation were kept without oxygen for periods of 7 and 19 hours, and 5 days, respectively, after which the supply of oxygen was restored. The controls grew in the presence of oxygen. All the plates were kept in continuous light.

Lack of oxygen caused a lower rate of growth of the fungus (absorption for 7 hours and 19 hours) or a cessation of growth (absorption for 5 days) and an interruption of spore formation. Renewed growth and sporulation took place when the supply of oxygen was restored, but in most cultures a spore-less ring, corresponding to the period of oxygen absorption, was apparent. This ring persisted even after all the remainder of the plate was covered by spores.

Mycelial growth seems to be affected only by much longer periods of oxygen withdrawal. After 5 days' absorption of oxygen the renewed mycelial growth was found to be fan-like and not peripheral. It is, therefore, assumed that some portions of the mycelium died due to the prolonged lack of oxygen.

From the above experiments it may be tentatively concluded that oxygen is essential for spore formation. Under the conditions of these experiments, the spore-forming ability of the mycelium seems permanently impaired where mycelial growth took place while oxygen was withdrawn, even if for short periods only.

DISCUSSION AND CONCLUSIONS

Exposure to light is known to be one of the factors which induce formation of spores or fruiting bodies in various fungi. In some laboratories it is a common routine to expose growing cultures to light in order to stimulate fructification.

The results of our experiments have shown clearly the influence light has on the sporulation of *Trichoderma viride* and on some cultural features of this fungus.

In our experiments daylight fluorescent lamps were used, the spectrum of which is almost identical with that of natural daylight (Paterson 1946). For technical reasons it was impossible to carry out experiments with monochromatic light so as to study the effect of different wavelengths on the results of the exposure. Such experiments may give rather interesting results, since various parts of the spectrum affect differently the metabolic processes in the fungus: the short wavelengths are generally (Castle 1931, Hawker 1950, Barnett and Lilly 1952, Miller, a. o. 1953, Tatarenko 1954, Sagromsky 1956), but not always (Lilly and Barnett 1951, p. 312), more active than the long ones. In those of our experiments where red light was used, it was found to have no perceptible effect on sporulation of *Trichoderma*. This observation is in agreement with those made by Barnett (1955) and Miller (1955).

The results of our experiments have shown that light may sometimes influence or even upset some morphological characters which were accepted as constant for taxonomic purposes, or it may sometimes stress previously unnoticed differences between isolates of *Trichoderma*.

The importance of light has hitherto been neglected in taxonomic studies and the conditions of illumination were not specified in morphological descriptions. But it should now be realized that the incubation of cultures in a thermostat (in complete darkness or sometimes intermittent monochromatic light) or in continuous light or on a laboratory shelf (in alternating diurnal light and darkness) create, diverse growing conditions, which may result in quite different morphological characters. It seems, therefore, advisable that light should be included as an important factor in all taxonomic studies. The possible morphological differences between the cultures, together with other inherent morphological characters and diversities in the physiology and pathogenicity of the cultures studied, may serve as convenient diagnostic tools for the identification of the organisms under investigation.

The extent to which sporulation is stimulated by light is determined by the intensity of the latter and by the length of exposure. The values obtained by us are of the same order as those of Miller (1955), who found that a one-minute exposure to a source of light of a one foot-candle intensity is sufficient to induce sporulation in *Trichoderma*.

Our experiments with various light intensities and different periods of exposure have shown that there is a correlation between these two factors, which determine the light energy, and the resulting sporulation. This is in conformity with the reciprocal time-intensity relationships prescribed by the Bunsen-Roscoe law (of 1863).

According to various workers, fungi differ in the conditions of light and darkness needed to attain the best sporulation or fructification: in some cases a period of darkness must follow the exposure (Barnett and Lilly 1950, Witsch and Wagner 1955), in others darkness must precede the illumination (in the dark a light-sensitive

substance is formed which is subsequently activated by light) (Hawker 1950), in yet other cases alternating light and darkness ensure best results (Timnick a. o. 1951). For *Trichoderma*, no such "dark period" is required to show up the effect of light on sporulation, since cultures grown in continuous light sporulated freely and abundantly.

Light, though having a striking effect on sporulation of *Trichoderma*, did not influence the mycelial growth rate, which remained equal in both light and darkness. Sagromsky (1956), working with *Trichothecium roseum*, reached the same conclusion, while Snyder and Hansen (1941) and Tatarenko (1954) found that cultures of *Fusarium* sp. and moulds (specia of *Penicillium* and *Aspergillus*), respectively, kept in the dark, showed greater mycelial growth than those kept in light.

According to the results of our experiments with *Trichoderma*, alternating light and darkness brought about the formation of concentric rings, whereas a single exposure resulted in one ring only. Hall (1933, cited after Hawker 1950), working with *Monilia fructigena*, has shown that one or more subsidiary ridge-like sporulating zones may develop as a result of one single exposure.

Our results further showed that no response to the stimulus of light can be evoked from spores at various stages of germination, nor from germination tubes, "old" hyphae, and mycelium that has already started to form spores. Thus, the sporulation-inducing effect of light is restricted to a definite stage in the development of the mycelium.

It may be assumed that at different portions along the hyphae, different (enzymatic?) processes take place or various metabolites accumulate. Sporulation may then be brought about by the effect of light on a metabolite at a specific point of the mycelium, or by a light induced change in some enzymatic processes.

Bisby (1925), Hall (1933, cited after Hawker 1950) and Snyder and Hansen (1941), working with fungi other than *Trichoderma*, found that only the marginal, actively growing parts of the colony, are susceptible to the stimulating effect of light. With *Trichoderma* the youngest parts of the hyphae do not respond to the effect of light. However, sporulation is induced in that part of the mycelium which is at a distance of 10—20 mm from the hyphal tip, thus effecting the above mentioned "shifts" (see Figure 1). This does not exclude the possibility of immediate photochemical reactions taking place at the hyphal tip; if so, the products of such reactions must then be transported to a more distant part of the mycelium where sporulation is induced. It is also possible that the stimulating effect of light is due to a hormone, but no experimental evidence has, to our knowledge, been adduced for this.

The culture medium used in this study was potato-dextrose agar. But the medium (its various nutrients and their amount) has been found by many workers (Barnett and Lilly 1950, Hawker 1950, (p. 189), Timnick a. o. 1951, Miller 1955, and Sagromsky 1956) to influence to some extent the results of illumination experiments. This aspect certainly deserves further study.

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REFERENCES

1. Barnett, H. L., 1955, private communication.
2. Barnett, H. L. and Lilly, V. G., 1950, Influence of nutritional and environmental factors upon asexual reproduction of *Choanephora cucurbitarum* in culture, *Phytopathology*, **40**, 80—89.
3. Barnett, H. L. and Lilly, V. G., 1952, The effect of color of light on sporulation of certain fungi, *Proc. W. Va. Acad. Sci.*, **24**, 60—64.
4. Bisby, G. R., 1925, Zonation in cultures of *Fusarium discolor sulphureum*, *Mycologia*, **17**, 84—97.
5. Castle, E. S., 1931, The phototropic sensitivity of *Phycomyces* as related to wavelength, *J. gen. Physiol.*, **14**, 701—711.
6. Hall, M. P., 1933, An analysis of the factors controlling the growth form of certain fungi, with special reference to *Sclerotinia* (*Monilia*) *fructigena*, *Ann. Bot.*, **47**, 538—578.
7. Harter, L. L., 1939, Influence of light on the length of the conidia in certain species of *Fusarium*, *Amer. J. Bot.*, **26**, 234—243.
8. Harter, L. L., 1941, The personal element and light as factors in the study of the genus *Fusarium*, *J. agric. Res.*, **62**, 97—107.
9. Hawker, L. E., 1950, *Physiology of Fungi*, University of London Press, London, pp. 360.
10. Houston, B. R. and Oswald, J. W., 1946, The effect of light and temperature on conidium production by *Helminthosporium gramineum* in culture, *Phytopathology*, **36**, 1049—1055.
11. Leonian, L. H., 1924, A study of factors promoting pycnidium formation in some *Sphaeropsidales*, *Amer. J. Bot.*, **11**, 19—50.
12. Lilly, V. G. and Barnett, H. L., 1951, *Physiology of the Fungi*, McGraw-Hill, N.Y., pp. 464.
13. Miller, J. J., 1955, private communication.
14. Miller, J. J., Webb, N. P. and Reid, J., 1953, Some experiments on the stimulation of sporulation in *Trichoderma lignorum* by radiation, Abst. in *Proc. Canad. phytopath. Soc.*, **20**, 19—20.
15. Paterson, C. C., 1946, Electric lighting by luminescence, *Endeavour*, **5**, 18—23.
16. Sagromsky, H., 1956, Zur lichtinduzierten Ringbildung bei Pilzen, III, *Biol. Zbl.*, **75**, 385—397.
17. Snyder, W. C. and Hansen, H. N., 1941, The effect of light on taxonomic characters in *Fusarium*, *Mycologia*, **33**, 580—591.
18. Tatarenko, E. S., 1954, Influence of light on growth of moulds, *Mikrobiologiya*, **23**, 29—33 (Russian).
19. Timnick, M. B., Lilly, V. G. and Barnett, H. L., 1951, Factors affecting sporulation of *Diaporthe phaseolorum* var. *batatatis* from soybean, *Phytopathology*, **41**, 327—336.
20. Witsch, H. v. and Wagner, F., 1955, Beobachtungen ueber den Einfluss des Lichtes auf Mycel- und Conidienbildung bei *Alternaria brassicae* var. *dauci*, *Arch. Mikrobiol.*, **22**, 307—312.

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